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by

Hiroaki Kamishina
I dedicate this work to my parents, Mr. Yoshio Kamishina and Mrs. Reiko Kamishina. Without their constant encouragement and support over the year, I would not be here documenting this thesis.
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Degenerative joint disease (DJD) of the hip in cats was investigated with non-invasive imaging techniques, gross and histopathology, and a three-dimensional culture system.

One hundred fifty-six cat cadavers collected from a local animal shelter were radiographically and grossly evaluated for degenerative changes of hip joints. Results of a radiographic and gross pathologic correlation study revealed a low sensitivity of radiography in detecting cartilage lesions. In radiographically normal 134 cats 28.4% of the cats (38/134) had gross lesions. On ventrodorsal radiographs, laterally pointed dorsocranial acetabular rims were seen in 10 cats. The cats with laterally pointed dorsocranial acetabular rims had a higher incidence of gross lesions (70.0%) than that of cats with flattened dorsocranial acetabular rims (24.7%). Therefore, the laterally pointed rims were commonly associated with hip DJD. The incidence of gross lesions in hip
cartilage was higher in castrated males (50.0%) than in intact males (22.9%). A higher incidence of gross lesions was found in adult cats (40.7%) compared to young cats (15.2%). Joint laxity measured with the Norberg angle was thought to be an important risk factor in hip DJD in cats. Hip dysplasia was diagnosed in 11 cats (7.1%) and commonly associated with advanced radiographic signs of hip DJD.

Magnetic resonance imaging (MRI) of feline hip joints was performed with high resolution (in-plane resolution of 230µm), using a 4.7-tesla MRI unit. A proton-density fat-suppressed fast spin-echo sequence was useful, especially in depicting a subchondral bone. In a joint with DJD, a thickened subchondral bone was clearly seen with this sequence, which was confirmed on a high-detail radiograph. Sodium MRI was unsuccessful in imaging feline hip joints most likely due to a low sensitivity of the used coil with respect to the intensity of the sodium signals from hip cartilage.

Feline chondrocytes were cultured in alginate microspheres at a density of 5x10^5/ml for 24 days. Results of DNA measurements showed that the chondrocytes did not proliferate until day 18. Newly synthesized chondroitin sulfates were extracted from the microspheres with equilibrium density centrifugation. The amounts of chondroitin sulfate-4 and chondroitin sulfate-6 were quantified and sulfation patterns were analyzed, using capillary electrophoresis. Only chondroitin sulfate-6 was detected on day 18 and 24. The effects of carprofen on chondrocyte proliferation and chondroitin sulfate synthesis were evaluated. Carprofen delayed cell death in a dose-dependent manner but the differences were not statistically significant compared to a control group. The positive effects of carprofen on chondroitin sulfate synthesis were not observed in this study.
CHAPTER 1
INTRODUCTION

Hip dysplasia has been described in cats. Degenerative joint disease (DJD) is also relatively common in geriatric cats. In an advanced stage, one can readily detect severe remodeling changes on radiographs, and the diagnosis can be readily made. However, companion cats do not show severe clinical signs due to DJD (osteoarthritis). This may be one of the reasons why extensive research has not been performed on this subject in cats. Radiographic signs or criteria for diagnosis for early DJD have not been well defined in the literature. Yet, veterinarians routinely face the situation to treat cats with lameness, presumably due to hip DJD, with non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying nutraceutical agents such as chondroitin sulfate and glucosamine. Thus, it is important to determine the normal variations in radiographic anatomy and the radiographic patterns of early DJD in the hip joints of cats.

Further, in recent years, other advanced imaging modalities such as magnetic resonance imaging (MRI) and computed tomography (CT) have become available to veterinarians as a non-invasive clinical imaging tool. However, the normal as well as degenerative changes of the hip or hip articular cartilage have not been studied in cats. Since these imaging modalities are expensive, it is warranted to describe the quality of images.

Lastly, feline articular chondrocytes have not been used for cell culture studies. In recent years, a three-dimensional chondrocyte culture system has been established. Since
there is no data on chondrocyte culture in cats, it is significant to test if culture can be done using feline articular cartilage.

This thesis is written in 3 chapters. Each chapter represents a single research report. In the second chapter, the radiographic and pathologic correlation of hip joints of cats is presented in special reference to degenerative changes. In the third chapter, a magnetic resonance imaging study (4.7-T) of the feline hip is described. In the last chapter, in vitro feline hip articular cartilage chondrocyte culture is described.
CHAPTER 2
DEGENERATIVE JOINT DISEASE OF THE HIP IN CATS: RADIOGRAPHIC AND PATHOLOGIC CORRELATION

Introduction

Degenerative joint disease (DJD) is a clinically significant disease in people (Felson 1990). When clinical signs are present, the condition is referred to as osteoarthritis (Resnick and Niwayama 1995). Similarly, DJD of the hip is a common radiographic abnormality in dogs and frequently associated with hip dysplasia (HD) (Smith et al., 1995, 2001). In cats, hip DJD has not been well studied, despite a few clinical reports on HD associated with clinical lameness (Holt 1978; Rabin et al., 1994; Patsikas et al., 1998).

Keller et al. (1999) reported that the frequency of feline HD was 6.6% (45/684) based on a survey of ventrodorsal abdominal radiographs. In the study, there was no statistical difference in the frequency of HD between breeds, but purebred cats showed a higher frequency than domestic cats (12.3% vs 5.8%, respectively). Females showed a higher frequency than males (8.8% vs 4.8%, respectively) but the difference was not statistically significant. Although these authors reported that the most common radiographic sign of HD was a shallow acetabulum (45/45) which was frequently seen in cats with DJD (43/45), a definition of the shallow acetabulum was not clearly made. The authors also stated that subluxation of the femoral head was not consistently associated with DJD. In this study, subluxation of the femoral head was subjectively evaluated (incongruent or non-parallel joint surfaces).
In a recent retrospective study of ventrodorsal abdominal radiographs of 88 cats (mean age of 9.2 years, ranging from 1.3 to 17.3 years), 60 of 88 (68.2%) cats showed radiographic signs of DJD in hip joints (Kamishina and Miyabayashi 2002). In this study, only radiographs with a well-positioned pelvis (determined by symmetrical obturator foramena and parallel femurs) were reviewed to accurately detect radiographic signs of hip DJD. The most common radiographic degenerative change was an osteophyte formation at the dorsocranial acetabular rim (57/60; 95.0%). An osteophyte formation on the femoral heads was also commonly detected (41/60; 68.3%), but an enthesophyte formation on the femoral neck was rarely seen (3/60; 5.0%). The radiographic pattern of the degenerative changes was different from the pattern described in dogs, which most commonly have femoral head and neck osteophytes (Morgan 1987; Smith et al., 1993). Furthermore, joint laxity was evaluated by measuring the Norberg angle (NA). The mean NA was significantly lower in cats with DJD (92.7° ± 8.5) than that in cats without DJD (97.9 ± 5.5). The mean NA significantly decreased with the severity of DJD, suggesting a relationship between joint laxity and a development of hip DJD in cats. The mean age of the cats with DJD (9.6 ± 4.1 years old; a range of 2.2 to 17.3) was higher than that of the cats without DJD (8.5 ± 4.2 years old; a range of 1.3 to 16.5). There was no statistical difference between these two groups. However, the joints were not evaluated grossly or histologically. Therefore, the authors could not confirm whether the osteophyte formation on the acetabular rim was indeed due to primary or secondary DJD or anatomical variations in cats.

In another recent radiographic survey, Hardie et al. (2002) reported the incidence of radiographic signs of DJD in geriatric cats (100 cats over 12 years old). In the study,
radiographic signs of DJD were graded based on the severity; grade 0 (normal joint),
grade 1 (a small enthesophyte or osteophyte), grade 2 (obvious enthesophyte or
osteophyte but minimally affected joint structure), and grade 3 (extensive peri- and intra-
articular mineralization, severe subchondral sclerosis, and joint remodeling). The authors
stated that 90% of the cats showed radiographic signs of DJD and neurologic signs
associated with lesions in the lumbosacral portion of the vertebral column. However, as
pointed out in a letter by Morgan and Pool (2002), the authors incorrectly included
degenerative disease of intervertebral joints which has a different pathogenesis than
degenerative diseases of synovial joints. The authors reported that the most commonly
and severely affected appendicular joint was the elbow (71 elbows were evaluated and
80% of the joints graded 2 or 3 were the elbow). However, other joints such as stifles (13
joints) and hips (18 joints) were not fully evaluated, suggesting a skewed sample
population.

Langenbach et al. (1998) reported a positive relationship between hip DJD and
joint laxity in 78 cats over 6 months of age. Radiographically, the hip joints were scored
as having excellent, good, fair, borderline, mild HD, moderate HD, and severe HD, based
on the criteria for canine HD as recommended by the Orthopedic Foundation for Animals
(OFA) (Corley et al., 1997). Joint laxity was evaluated by measuring the NA.
Distraction index (DI) was also measured, using a PennHip procedure as described for
dogs (Smith et al., 1990). The NA was lower in cats with DJD (84 ± 10°, ranging form
56 to 100°) than ones without DJD (95 ± 5°, ranging from 78 to 105°). The mean DI in
cats with hip DJD (0.6 ± 0.11, ranging from 0.40 to 0.80) was significantly higher than
that in cats with normal hip joints (0.49 ± 0.14, ranging from 0.20 to 0.84). Furthermore,
cats with a DI less than 0.4 did not show radiographic signs of DJD. Hip dysplasia was found in 25 cats (32%) based on the OFA-like scoring system. Of 25 cats with HD, 15 cats had radiographic signs of DJD. A high incidence of hip DJD was noted in the moderate (100%) and severe HD (100%) groups compared to the mild HD group (47.3%). In this study, the hip joints were evaluated based on the scoring system established for dogs. This may have led authors to overestimate HD in the cats, since cats seemed to have more laxity in hip joints as demonstrated by the lower NA (95°) than those of dogs (established normal lower limit of 105°) (Douglas and Williamson 1970). In addition, a relationship between DJD and age, body weight, and clinical history was not assessed in this study.

The purposes of the present study were 1) to establish a radiographic appearance of normal hip joints and hip joints with DJD and 2) to investigate clinical radiographic, high-detail radiographic, gross pathologic, and histopathologic changes associated with feline hip DJD in a special reference to the pointed appearance of the dorsocranial rim on the ventrodorsal radiographs of hips.

**Material and Methods**

**Cat Cadavers**

A total of 181 cat cadavers (referred to as cats throughout this thesis) were collected from a local animal shelter immediately after euthanasia. Body weight, breed, and gender were recorded. Male cats were recorded as intact or castrated, while no attempt was made to separate females into intact and spayed. Age was not known for any of the cats. However, a lateral whole-body radiograph was made to exclude skeletally immature cats from the study. The cats were excluded if the distal tibial and/or proximal ulnar physis were open. The lateral whole body radiographs were also used to evaluate
other appendicular joints to exclude the cats with radiographic signs of polyarthropathy such as subchondral bone destruction and indistinct periarticular new bone formation in multiple joints. The included cats were then divided into two age-related groups (young and adult) based on the following criterion. Cats with open proximal humeral physis were grouped into the young group. If the proximal physis of the humerus was closed, the cats were grouped into the adult group.

The use of these cats was approved by the Institutional Animal Care & Use Committee at the University of Florida.

Radiographic Examination

A ventrodorsal radiograph of the pelvis was taken, using a clinical radiographic machine (Innovet, Summit industries Inc, Chicago, IL). All cats were placed in a trough in dorsal recumbency to obtain a radiograph with a symmetric pelvis. The hind limbs were adducted and extended parallel to the vertebral column. This method was adopted from the radiographic standard for submission of hip evaluation in cats at OFA.

Each radiograph was interpreted regarding an osteophyte formation at a dorsocranial acetabular rim, an enthesophyte formation, an osteophyte formation on a femoral head and neck, and a sclerotic change in an acetabular fossa. Based on the size of the new bone formation or osteophytes on the acetabular rim, acetabular fossa, femoral head, and femoral neck, each joint was graded as normal (no osteophyte), mild DJD (osteophyte less than 1mm), moderate DJD (less than 2mm), and severe DJD (more than 2mm) from at least one anatomical area. Norberg angles were measured in all joints as previously described (Douglas and Williamson 1970).

Hip dysplasia was diagnosed if the joints showed subluxation with or without degenerative changes.
The obesity index was calculated, using the following formula; Obesity index = body weight (kg) / total length (cm) of lumber vertebrae.

**Stress Radiography**

Stress radiographs of the hip joints were added in 65 of 154 cats as previously described by Fluckiger et al. (1999) in dogs. Briefly, the cats were placed in a trough in dorsal recumbency. The stifles were adducted and the femurs were positioned at approximately 60° angle to the table. A force was manually applied to the hip joints, so that dorsocranial and lateral subluxation occurred in loose hips. The force was evenly applied to both hip joints. The subluxation index, which is similar to the DI described by Smith et al. (1990), was calculated as follows; The distance between the center of the femoral head and center of the acetabular fossa was divided by the radius of the femoral head.

**Gross Pathologic Examination**

After the radiographic examination, the femoral heads and acetabula were excised, using a handsaw. Articular cartilage of the femoral heads and acetabula was examined for gross abnormalities under a stereo microscope (SZ4045, Olympus Optical Co., Ltd. Tokyo, Japan). The articular cartilage was graded as normal (intact articular cartilage), mild DJD (surface dullness or irregularity), moderate DJD (surface irregularity and/or a partial loss of articular cartilage), and severe DJD (a complete loss of articular cartilage and subchondral bone exposure). Lesions were localized in a cranial, caudal, or dorsal segment of the femoral head or acetabulum.

**High-detail Radiography**

Randomly selected specimens of the femoral heads and acetabula from each group based on the radiographic grades were serially sectioned transversely at 2-3 mm intervals,
using a low speed saw (Isomet 11-1180, Buehler Ltd, Evanston, IL) with a diamond-coated blade (Diamond Wafering Blade 11-4245, Buehler Ltd, Evanston, IL). High-detail radiographs were made, using a cabinet radiographic machine (Faxitron Model MX-20, Faxitron X-Ray Corporation, Wheeling, IL) with non-screen films (Kodak X-Omat TL, Eastman Kodak Company, Rochester, NY). The following technique was used for all specimens: 30 kVp, 0.3 mA, and 60 sec, at 57.2 cm FFD. High-detail radiographs were evaluated for the presence of an osteophyte formation, enthesophyte formation, subchondral sclerosis, and a trabecular pattern. The specimens were then placed in a neutral-buffered 10% formalin solution for the histopathologic examination.

Histopathologic Examination

After the gross and high-detail radiographic evaluations, the selected specimens were further evaluated histologically from each grade group. Thin-section specimens were decalcified in a neutral-buffered 25% formic acid solution under gentle agitation. Duration of the decalcification process was determined based on high detail radiographs. After this process, bones were rinsed in running tap water, dehydrated in a graded series of ethanol, and embedded in paraffin. Embedded specimens were sectioned in 5 microns with a microtome and stained with H & E and the Safranin-O staining.

Statistical Analyses

Two sets of data were made. For the first data set, cats were grouped based on the radiographic grades. For the second data set, cats were grouped based on the gross pathologic grades. Because of the small number of purebred cats, all purebred cats were pooled into one group for comparison purposes. Comparison of genders was performed between males and females as well as between intact males and castrated males.
To perform the following statistical analyses, the grades from both sides of the joints were combined. When cats showed asymmetrical grade, the highest grade was selected. To examine the difference of the incidence of hip DJD between breeds (domestic vs purebred cats), genders, and the two age-related groups, Chi-square test or Fisher’s exact test was used (SAS Version 6.12).

For the following statistical analyses, each joint was tested individually. Among the four groups (normal, mild, moderate, and severe) either based on the radiographic grades or the gross pathologic grades, body weight, obesity index, NA, and subluxation index were compared with One-way analysis of variance (ANOVA) (SAS Version 6.12). Since cats with HD were expected to have low NA, comparisons of NA were performed on three groups; (1) all cats, (2) cats without HD, and (3) cats with HD. If significant differences were detected with One-way ANOVA, Tukey’s studentized range test was performed to determine the significant differences among the tested groups (SAS Version 6.12). A value of p<0.05 was considered significant.

Results

Sample Population

A total of 181 cats were collected and 25 cats were excluded from the study. Of those excluded, 24 were considered to be too immature (based on the lateral whole body radiographs) and 1 had a fracture of the ilium. No cat showed radiographic evidence of polyarthritis. There were 140 domestic cats and 16 purebred cats including 11 Siamese, 3 Main coon cat, 1 Russian Blue, and 1 Persian cat. There were 78 males (48 were intact) and 78 females. There were 33 cats in the young group and 123 cats in the adult group. The mean ± SD body weight was 3.83 ± 0.94 and ranged from 1.8 to 6.1 kg. Genders, breeds, body weights, and age-related groups were summarized (Table 2-1).
Radiographic Findings

A total of 312 hip joints from 156 cats were evaluated for the radiographic signs of DJD. The overall incidence of hip DJD was 14.1% (22/156). Based on the radiographic grades, there were 134 cats (85.9%) with normal hip joints, 14 cats (9.0%) with mild DJD, 6 cats (3.8%) with moderate DJD, and 2 cats (1.3%) with severe DJD, respectively. Hip DJD was seen unilaterally in 8 cats; 7 cats had mild DJD on one side, and 1 cat had moderate DJD on one side. Bilateral hip DJD was found in 14 cats; 7 cats with mild DJD on both hip joints, 4 cats with mild and moderate DJD, 1 cat with moderate DJD on both joints, 1 cat with moderate and severe DJD, and 1 cat with severe DJD on both joints.

The most common radiographic sign was the osteophyte formation at the dorsocranial acetabular rims. The overall frequency of this radiographic finding was 13.5% (21/156). In each group, the frequency of this finding was 92.9% (13/14), 100% (6/6), and 100% (2/2) in the mild, moderate, and severe DJD groups, respectively.

On the ventrodorsal radiographs, the dorsocranial aspects of the acetabular rims varied in shape (Figures 2-1-A and 2-1-B). Dorsocranial acetabular rims showed either a flattened or laterally pointed appearance. Laterally pointed dorsocranial acetabular rims were found in 10 cats (6.4%; 10/156). The contours of the rims were distinct in 8 cats and considered normal (Figure 2-1-B). The contours of the acetabular rims were indistinct in 2 cats and small osteophytes were observed (Figure 2-1-C). In cats with moderate and severe DJD, enlarged osteophytes were clearly noted (Figure 2-1-D).

A sclerotic change in the acetabular fossa was seen in 9 cats (5.1%). The frequency of this finding in each group was 21.4% (3/14), 66.7% (4/6), and 100% (2/2) in the mild, moderate, and severe DJD groups, respectively. Of these cats having sclerotic changes in the acetabula, 88.9% (8/9) of the cats had osteophyte formation at the acetabular rims.
The femoral heads and necks less frequently showed osteophytes than acetabula did. The overall frequency of this finding was 1.9% (3/156). There was only 1 cat with this finding in each group (mild, moderate, and severe DJD).

Eleven cats (7.1%) were considered to have HD: 10 females and 1 male. There were 9 domestic cats, 1 Siamese, and 1 Persian cat. Hip dysplasia was bilateral in all cats. Of 11 cats with HD, 5 cats had no radiographic sign of DJD, while 4 cats had moderate DJD and 2 cats had severe DJD (Figure 2-1-D). Of 6 cats with hip HD, all cats showed osteophyte formations on the dorsocranial aspect of the acetabular rims. Sclerotic changes in the acetabular fossa were much more commonly seen among the cats with HD (83.3%) than the cats without HD (36.4%).

Breeds, genders, and the age-related groups were assessed in relation to the radiographic incidence of hip DJD. Twenty of 140 domestic cats (14.3%) showed radiographic signs of hip DJD, whereas 2 of 16 purebred cats (12.5%) showed DJD. Ten of 78 males (12.8%) showed DJD and 12 of 78 females (15.4%) showed DJD. Of 10 males, 6 were intact and 4 were castrated. In the young group, there were 2 cats (6.1%) with DJD, whereas there were 20 cats (16.3%) with DJD in the adult group. There was no statistical difference in the incidence of radiographic signs of DJD between breeds, genders, and the age-related groups. The results were summarized (Table 2-2).

Among the groups based on the radiographic grades, the differences of body weight, obesity index, subluxation index, and NA were assessed. Since there were only 2 cats in the severe group the moderate and severe groups were combined. The mean ± SD body weight (kg) in the normal, mild, and moderate/severe groups were 3.8 ± 0.9, 4.3 ± 0.9, and 3.6 ± 1.3, respectively. The mean body weight in the mild group was
significantly higher than that of the normal group. The mean ± SD obesity index were 0.27 ± 0.06, 0.31 ± 0.06, and 0.26 ± 0.07, in the normal, mild, and moderate/severe groups, respectively. The mean obesity index was statistically higher in the mild group than those of the normal and moderate/severe groups. The mean ± SD subluxation index were 0.25 ± 0.14, 0.42 ± 0.29, and 0.32 ± 0.17 in the normal, mild, and moderate/severe groups, respectively. The mean subluxation index was significantly higher in the mild group than that of the normal group. The mean subluxation index was higher in the moderate/severe group than the normal group and lower than the mild group without statistical differences. The results were summarized (Table 2-3).

When the all cats were included, the mean ± SD NA were 100.7 ± 7.0, 93.8 ± 8.7, and 88.3 ± 8.4 in the normal, mild, and moderate/severe groups, respectively. The mean NA significantly decreased in the mild and moderate/severe DJD groups from the normal group. The difference of the mean NA was not significant between the mild and moderate/severe DJD groups. When the cats without HD were compared, the mean ± SD NA were 100.9 ± 6.9, 96.0 ± 7.3, and 96.7 ± 1.5 in the normal, mild, and moderate/severe groups, respectively. The mean NA was significantly lower in the mild group than that of the normal group. When the cats with HD were compared, the mean ± SD NA angle were 94.0 ± 3.1, 82.3 ± 6.4, and 85.2 ± 7.7, in the normal, mild, and moderate/severe groups, respectively. The mean NA was significantly lower in the mild and moderate/severe groups compared to that of the normal group. The results were summarized (Table 2-4).

**Gross Pathologic Findings**

Gross pathologic examinations were performed on 312 joints from 156 cats under a stereo microscope. One hundred one cats (64.7%) had grossly normal articular cartilage
on the femoral heads and the acetabula. Normal femoral heads and acetabula had a smooth and glistening layer of articular cartilage (Figures 2-2-B and 2-4-B). On the caudal aspect of the femoral articular cartilage a localized region with surface dullness was noted, mimicking an early degenerative change of the articular surface. This finding was noted in 148 femoral heads (47.4%). Of 148, 126 were radiographically normal, 18 were graded as mild DJD, and 4 were graded as moderate DJD. On the other hand, of 141 femoral heads without this finding, 132 were radiographically normal, 6 were graded as mild DJD, 1 was graded as moderate DJD, and 2 were graded as severe DJD. A similar change was also noted on the dorsocaudal periphery of the acetabular cartilage (24.4%; 76/312) (Figure 2-6-B). However, on the histological examination, it was noted that these areas were partially composed of fibrous cartilage and slight surface irregularity may be present in normal cartilage. Therefore, if dullness of these areas was the only change noted, it was considered to be a normal variation and not grossly graded as degenerative changes.

Gross abnormalities of articular cartilage were found in 55 cats (35.3%). Of 55 cats, the lesions were bilateral in 30 cats, whereas unilateral lesions were found in 25 cats.

Of 312 femoral heads examined, 249 were normal (79.8%), whereas 38 (12.2%) had mild, 23 (7.4%) had moderate, and 2 (0.6%) had severe gross lesions. With mild DJD, the lesions were seen as focal dullness of the articular surface. Those lesions were found on the dorsal aspects in 43 femoral heads. Mild gross lesions on the cranial aspect were found in 17 femoral heads. Generalized dullness of the articular surface was also seen mostly with advanced changes (Figures 2-8-B and 2-8-C). Moderate lesions (partial
loss of cartilage) were found on the caudal aspects in 21 femoral heads (Figure 2-8-C), on the dorsal aspects in 2 femoral heads, and on the cranial aspects in 3 femoral heads. Severe lesions (complete loss of cartilage) were seen only in 2 femoral heads, which were both on the caudal aspects.

Of 312 acetabula examined, 270 (86.5%) were normal, whereas 25 (8.0%) had mild lesions and 17 (5.4%) had moderate lesions. A severe lesion was not found on the acetabula. Mild gross lesions were found on the dorsal aspects in 23 acetabula and on the cranial aspects in 21 acetabula. Moderate gross lesions were found on the caudal, dorsal, and cranial aspects in 14, 2, and 8 acetabula, respectively.

Breeds, genders, and the age-related groups were assessed in relation to the incidence of the gross lesions. The gross lesions were found in 49 of 140 domestic cats (35.0%) and in 6 of 16 purebred cats (37.5%). The difference was not significant.

Female cats (29/78; 37.2%) had a higher incidence of gross lesions than male cats did (26/78; 33.3%) without a statistical difference. The incidence of gross lesions in castrated males (50.0%) was significantly higher than that of intact males (22.9%).

The adult group (40.7%) had a significantly higher incidence of the gross lesions compared to the young group (15.2%). The results were summarized (Table 2-5).

Among the groups based on the gross pathologic grades, the differences of body weight, obesity index, subluxation index, and NA were assessed. Since there was only 1 cat in the severe group the moderate and severe groups were combined. The mean ± SD body weight (kg) in the normal, mild, and moderate/severe groups were 3.9 ± 0.9, 3.9 ± 1.1, and 3.7 ± 1.0, respectively. The mean ± SD obesity index were 0.27 ± 0.05, 0.28 ± 0.07, and 0.26 ± 0.07 in the normal, mild, and moderate/severe groups, respectively.
There were no statistical differences in the mean body weight and obesity index among the three groups. The mean ± SD subluxation index were 0.27 ± 0.15, 0.28 ± 0.19, and 0.21 ± 0.12 in the normal, mild, and moderate/severe groups, respectively. The differences were not significant. The results were summarized (Table 2-6).

When the all cats were included the mean ± SD NA were 99.7 ± 7.0, 98.9 ± 10.0, and 100.3 ± 8.5 in the normal, mild, and moderate/severe groups, respectively. When the cats without HD were compared, the mean ± SD NA were 100.9 ± 6.9, 96.0 ± 7.3, and 96.7 ± 1.5, in the normal, mild, and moderate/severe groups, respectively. When the cats with HD were compared, the mean ± SD NA were 90.8 ± 6.4, 80.0 ± 7.7, and 89.8 ± 5.8 in the normal, mild, and moderate/severe groups, respectively. In cats with HD, the mean NA in the mild group was significantly lower than that of the normal group. The results were summarized (Table 2-7).

**Correlation: Radiographic Findings and Gross Pathologic Findings**

A correlation between radiographic and gross pathologic findings was evaluated. Of radiographically normal 134 cats, 96 (71.6%) were normal on the gross pathologic examinations, while 38 (28.4%) had gross lesions on the femoral heads or acetabula or both. Of 14 cats with radiographic signs of mild DJD, 3 (21.4%) were normal, while 9 (64.3%) had mild gross lesions, 1 (7.1%) had moderate gross lesions, and 1 (7.1%) had severe gross lesions. Of 6 cats with radiographically moderate DJD, 1 was normal, while 2 and 3 were mild and moderate on the gross examinations. Of 2 cats with radiographically severe DJD, 1 was normal and the other cat had a moderate gross lesion. The results were summarized (Table 2-8).

Of 10 cats with laterally pointed dorsocranial acetabular rims, 3 were grossly normal, whereas 5 had moderate gross lesions and 2 had mild lesions on the femoral
heads or acetabula. The incidence of the gross lesions was higher in the cats with pointed acetabular rims (70.0%; 7/10) than the cats with flattened acetabular rims (30.0%; 3/10).

**High-detail Radiographic Findings**

High-detail radiographs of the thin section specimens of the femoral heads and acetabula were evaluated to depict the degenerative changes in depth. Radiographically and grossly normal femoral heads showed uniform thickness of subchondral bone underlying articular cartilage except for the region around the attachment of the round ligament, which was thicker than other regions (Figure 2-2-D). On the caudal aspect of the femoral heads, a small ridge was noted with various sizes (Figure 2-2-D).

Subchondral bone was also thick under the ridge. The ridge located laterally to the caudal edge of articular cartilage and medially to the trochanteric fossa. Thickness of the subchondral bone of acetabula was difficult to evaluate due to concavity of acetabula.

Femoral heads with mild gross lesions showed no detectable abnormalities on the high-detail radiographs. However, in a femoral head with moderate gross lesions a thickened subchondral bone was seen at the caudal aspect of the femoral head (Figure 2-9-B).

In acetabula with mild gross lesions small osteophytes on the dorsal rim (articular lipping) and on the ventral rims were observed (Figure 2-6-D).

**Histopathologic Findings**

Radiographically and grossly normal articular cartilage of the femoral heads and acetabula showed a characteristic appearance of hyaline cartilage. In a superficial zone thin layers of densely packed chondrocytes that lined parallel to the articular surface were noted (Figure 2-3-A). On the Safranin-O stain, the superficial zone showed low stainability, suggesting an inherent low concentration of proteoglycans (Figure 2-3-B). Underneath the superficial zone where the zone called transitional zone, chondrocytes
were more evenly distributed. The Safranin-O stain demonstrated a high concentration of proteoglycans in this zone. The third zone, a radial or deep zone, contained chondrocytes that were organized in columns. High stainability of Safranin-O was noted especially around the chondrocytes (pericellular region). At the caudal aspect of the femoral cartilage where close to the lateral margin, hyaline cartilage was gradually replaced with fibrous cartilage from the surface area (Figure 2-3-C). The articular surface of this portion appeared slightly roughened. In addition, chondrocytes appeared less organized. On the Safranin-O stain, less stainability of the superficial zone and the upper part of the transitional zone was evident, suggesting a low concentration of proteoglycans (Figure 2-3-D). A similar pattern was noted on the dorsal periphery of acetabula, that is replacement of hyaline cartilage with fibrous cartilage and a low concentration of proteoglycans at this region (Figures 2-5-A and 2-5-B).

Histopathologic changes were seen on the femoral heads and acetabula. Femoral heads with moderate gross lesions showed surface roughening, hypocellularity, and diminished stainability on the H & E stain (Figure 2-10-A). On the Safranin-O stain, loss of proteoglycans from the upper part of the transitional zone was confirmed (Figure 2-10-B). With a grossly severe lesion, loss of cartilage and subchondral bone thickening were noted on the femoral head.

On the acetabula with mild gross lesions, an irregular surface of cartilage and loss of the superficial zone were noted on the H & E stain (Figure 2-10-C). Low stainability on the Safranin-O stain was also evident on the upper transitional zone and extended ventrally (Figure 2-10-D).
Discussion

In the present study, well-positioned hip joints were radiographically and grossly evaluated in 156 cat cadavers to establish a radiographic appearance of normal hip joints and hip joints with DJD.

The overall incidence of radiographic signs of hip DJD was 14.1% (22/156), which was much lower than that reported in a previous study (68.2%) (Kamishina and Miyabayashi 2002). The discrepancy was most likely due to the difference of the cat population between the two studies. In the present study, the exact ages of the cats could not be determined; however, based on the appearances of the lateral whole-body radiographs, the cats in this study seemed to represent relatively young cats. In addition, 33 of 156 cats (21.2%) were estimated to be younger than 2 years old (cats in the young group). In contrast, in the previous study, cats represented relatively old cats (mean ± SD age of 9.2 ± 4.1, ranging from 1.3 to 17.1). With aging, it has been well known that biochemical changes such as decreased proteoglycan contents and a change of glycosaminoglycan (GAG) composition occur in the cartilage matrix and play an important role in the development of primary DJD (Hardingham and Bayliss 1990; Malemud 1991). Similar changes have been reported in various species, including human (Bayliss et al., 1999), horses (Brown et al., 1998), cows (Murata and Bjelle 1980), and dogs (Harab and Mourao 1989). Although these biochemical changes in the cartilage matrix have not been investigated in cats, aged cats might have a high incidence of primary DJD as seen in other species.

We found that the cats with laterally pointed dorsocranial acetabular rims had a higher incidence (70.0%) of gross lesions on the femoral and acetabular cartilage than the cats with flattened acetabular rims (24.7%). Consequently, the laterally pointed
dorsocranial acetabular rims were thought to be one of early radiographic signs of hip DJD in cats. Nevertheless, 30% of the cats (3/10) with laterally pointed dorsocranial acetabular rims did not have gross lesions in hip joints. Since a histological evaluation was not performed on these acetabula there was a possibility that the pointed acetabular rims represent normal variations in some cases. In clinical cases, serial radiographic examinations should be performed to clarify this point.

The radiographic pattern of hip DJD was similar to those of previous studies (Keller et al., 1999; Kamishina and Miyabayashi 2002). The osteophyte formation on the dorsocranial acetabular rims was the most common radiographic sign and found in 95.5% (21/22) of the cats with hip DJD. In contrast, femoral heads and necks less frequently showed osteophytes (3/22; 13.6%). A similar pattern was reported in cats with hip DJD secondary to HD. In 3 cats with HD, dorsocranial acetabular rims showed marked new bone formations on radiographs, while only mild flattening of the femoral heads was detected on a gross examination (Patsikas et al., 1998). Holt et al. (1978) also reported that in a cat with HD, although femoral heads were macroscopically flattened histopathologic abnormalities were not found on femoral cartilage. These patterns were different from that of dogs where enthesophyte formation on a femoral neck was reported to be an early sign of hip DJD secondary to HD (Morgan 1987). The difference of the radiographic patterns of hip DJD between dogs and cats could be attributed to the difference of a body weight. Dogs are usually heavier than cats and intense mechanical stress may cause an enthesophyte formation that is detectable on radiographs at relatively early stages. In contrast in cats, surrounding soft tissues could be possible to support the
joints from the altered mechanical stress, owing to the less intense stress in the joints of cats compared to dogs.

Interestingly, cartilage lesions were more commonly found on the femoral heads (20.2%; 63/312) than acetabula (13.5%; 42/312) despite the higher incidence of radiographic signs of DJD on the acetabulum than that of femoral head. The exact reason why more gross abnormalities were detected on the femoral heads than acetabula was not clear. However, the structural difference between a femoral head and acetabular fossa could play a role; that is a femoral head has a convex structure, whereas an acetabulum has a concave structure. A similar tendency has been reported in the canine shoulder, which has a concave glenoid cavity and a convex humeral head. Ljunggren and Olsson (1975) investigated DJD in 88 canine cadavers that were euthanized at a veterinary teaching hospital for various reasons. The authors found cartilage lesions in 28 of the 88 dogs in the shoulder joints. The most common macroscopic finding was the cartilage erosions, which were located caudally in the center of the humeral head. There was a similar change in the glenoid surface of the scapula, but the changes were less pronounced than those in the humeral head. In a study by Tirgari and Vaughan (1973), 12 canine cadavers were evaluated for shoulder DJD. Erosions of humeral cartilage were commonly and invariably found in the center of the posterior part of the humeral cartilage; however, cartilage lesions were uncommonly found in the glenoid cavity.

Moreover, in dogs with HD, Farese et al. (1998) reported that when joint subluxation was present, load-bearing portions of a femoral head and acetabulum shifted to the perifoveal region on the femoral head and to the dorsolateral edge of the acetabular rim. The authors also demonstrated the lunate surface of the acetabulum, which lost its
concavity in dogs with HD. In cats, similar changes could occur in subluxated hip joints and femoral cartilage could be more susceptible to wear and tear, since pointed acetabular rims rub the articular surface of the femoral cartilage.

One of the remarkable findings in the present study was that the gross lesions were commonly located on the caudal aspect of the femoral head and acetabulum. Although the caudal segments on the femoral and acetabular cartilage were considered to be non-load bearing portions in dogs, cats may have different load-bearing portions on the femoral and acetabular cartilage. Kinematics of feline hip joints has not been studied but it appears to be different from that of dogs since cats have different behavior in terms of locomotion (i.e. often jump on a counter). We found that the caudolateral edge of the femoral cartilage and the dorsocaudal periphery of the acetabular cartilage were partly composed of fibrous cartilage. The caudal segments of femoral and acetabular cartilage could be more prone to wear and tear because of its imperfect composition as hyaline cartilage in cats. In fact, advanced gross lesions (moderate and severe lesions) were more commonly located on the caudal segment on the femoral head; 21 of 26 moderate lesions and 2 of 2 severe lesions were present on the caudal segments.

Based on the correlation between radiographic findings and gross pathologic findings, 28.4% of the cats with radiographically normal hip joints had gross lesions on hip cartilage. This result demonstrated a low sensitivity of radiography in detecting cartilage lesions in hip joints. The incidence of gross lesions increased with the severity of radiographic signs. The incidence of the gross lesions was 78.6% and 83.3% in the cats with mild and moderate radiographic signs, respectively. There were 2 cats that showed moderate and severe radiographic signs with no gross abnormalities. In these 2
cats, severely thickened joint capsule was observed. Thickened joint capsule might have reinforced or stabilized the joints, but the precise reason was not known.

Of evaluated risk factors, the mean body weight and obesity index of the cats in the radiographically mild DJD group were significantly higher than those of the normal group. However, the mean body weight and obesity index were lower in the moderate/severe group compared to the mild group. Based on the appearance of the lateral whole-body radiographs, the moderate/severe group seemed to be composed of relatively aged cats. Since geriatric cats tend to lose weight this might be why the mean body weight and the mean obesity index decreased in the moderate/severe groups.

The incidence of gross lesions in the castrated males (50.0%) was significantly higher than that of the intact males (22.9%). In a recent study, McNicholas et al. (2002) reported a higher incidence of spontaneous femoral capital physeal fractures in castrated male cats compared to intact males. In the study, 26 cats over 1 year old at the time the femoral capital fracture occurred and with no history of trauma were included. Twenty-five cats were castrated males and one was spayed female. Of 16 cats for which age at the time of neutering was known, 14 cats had been neutered before 6 months of age. Of 7 cats evaluated histologically, open femoral capital physes were observed in all specimens with physeal chondrocytes that clustered and lacked normal columnar arrangement. The delayed physeal closure was thought to result from early castration and caused the physeal fractures. In addition, the mean body weight of these cats was significantly higher than that of age- and sex-matched cats, suggesting the increased body weight as a part of the cause of fractures. In the present study, although the time of castration of the cats was not known, developmental abnormalities of the femoral heads might have
occurred, resulting in altered load-bearing of the joints. The castrated males also had a significantly higher mean body weight than that of the intact males (data not shown). Increased body weight might also have played a role in the development of the gross lesions in hip joints of castrated males.

In a previous study, the mean NA was significantly lower in cats with radiographic signs of DJD (92.7°) than cats with normal hip joints (97.9°) (Kamishina and Miyabayashi 2002). In another study, the mean NA in cats with radiographic signs of DJD was 84°, whereas that in cats with normal hip joints was 95° (Langenbach et al., 1998). Moreover, in the same study, the mean DI in cats with DJD (0.6) was significantly higher than that in cats without DJD (0.49). In the present study, the mean NA was significantly lower in cats with radiographic signs of DJD (92.1°) than that in cats with normal hip joints (100.7°). When the cats with HD were excluded from the analysis the mean NA of the mild group (96.0°) was significantly lower compared to the normal group (100.7°). The mean NA of the moderate/severe group (96.7°) was also lower than that of the normal group and the difference was nearly significant. These results supported that joint laxity was the significant factor in the development of hip DJD in cats as in dogs.

On the other hand, the NA was not significantly associated with the incidence and severity of gross lesions on hip cartilage when all cats were included in the comparison. Furthermore, the mean NA in cats with the caudal cartilage lesions was higher (100.2 ± 7.0°) than that in cats with the dorsal cartilage lesions (98.6 ± 9.8°) that typically resulted from joint laxity. This suggested that the gross lesions on the caudal aspects of the
femoral and acetabular cartilage could develop without joint laxity. This might be the reason why the weak association between the NA and gross lesions was found.

When only cats with HD were compared, cats with mild gross lesions had significantly lower NA (80.0°) than cats with normal hips (90.8°). However, the mean NA of cats in the moderate/severe group (89.8°) was not significantly different from that of cats in the normal group. One possible reason was that the thickened joint capsule associated with moderate and severe DJD tightened the joints, increasing the NA.

Hip dysplasia is a well-known cause of secondary hip DJD in dogs (Smith et al., 1995). Similarly, HD has been reported to be a significant cause of hip DJD in cats. However, the reported prevalence of HD in cats varied among studies most likely due to different definitions of HD among those studies. Keller et al. (1999) reported the incidence of HD in cats to be 6.6%, based on a radiographic survey of 684 cats. In the study, a definition of HD was not clearly stated. The authors subjectively diagnosed HD and made notations as to the radiographic findings suggestive of HD (subluxation, shallow acetabulum, remodeling of the femoral head/neck, acetabular rim changes and DJD). The incidence of HD in another study was 32% (25/78) (Langenbach et al., 1998). In the study, the diagnosis of HD was based on the OFA-like scoring system which was developed to diagnose canine HD. Therefore the authors might have overestimated HD in cats that seemed to have looser congruity in the hip than dogs. In the present study, HD was diagnosed when cats showed joint subluxation. The incidence of HD was 7.1% (11/156), which was close to the incidence reported by Keller et al. (1999). Furthermore, it was evident that the cats with HD showed more severe
radiographic signs of hip DJD than cats without HD. Therefore, HD should be considered as a significant cause of severe hip DJD, especially in young cats.

There were some limitations in the present study. Since all cats were collected from an animal shelter the exact ages of the cats were unknown. Since age is one of the important factors of DJD, especially in primary DJD, a longitudinal study is needed to describe age-related radiographic and gross pathologic findings of feline hip DJD.

The cats in this study were cadavers. This may have affected the results, possibly the NA and subluxation index measurements. However, the cats were radiographed within approximately one hour after euthanasia; therefore, postmortem changes were expected to be minimum. Even under the presence of postmortem changes, those changes should not have affected the radiographic appearances of the hip joints.

Clinical histories of the cats were also unknown. Having a relatively young cat population in this study, the incidence of hip DJD should be considered to be significantly high, particularly the incidence of gross lesions of 35.3%. Cats with cartilaginous lesions were likely to have clinical signs such as lameness and pain. Therefore, a study that associates the extent of the radiographic signs and clinical signs is warranted to determine the clinical significance of the findings derived from the present study.
Table 2-1 Sample population.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Breed</th>
<th>Age-related groups</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Domestic</td>
<td>140</td>
<td>Young</td>
</tr>
<tr>
<td>Intact</td>
<td>Purebred</td>
<td>16</td>
<td>Adult</td>
</tr>
<tr>
<td>Castrated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2-2 Frequency of radiographic signs of hip DJD between breeds, genders, and the age-related groups.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Normal</th>
<th>DJD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic</td>
<td>120 (85.7)</td>
<td>20 (14.3)</td>
</tr>
<tr>
<td>Purebred</td>
<td>14 (87.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Male</td>
<td>68 (87.2)</td>
<td>10 (12.8)</td>
</tr>
<tr>
<td>Intact</td>
<td>42 (87.5)</td>
<td>6 (12.5)</td>
</tr>
<tr>
<td>Castrated</td>
<td>26 (86.7)</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td>Female</td>
<td>66 (84.6)</td>
<td>12 (15.4)</td>
</tr>
<tr>
<td>Young</td>
<td>31 (93.9)</td>
<td>2 (6.1)</td>
</tr>
<tr>
<td>Adult</td>
<td>103 (83.7)</td>
<td>20 (16.3)</td>
</tr>
</tbody>
</table>

( ) indicates percentage.

Table 2-3 Body weight, obesity index, and subluxation index among the groups based on the radiographic grades.

<table>
<thead>
<tr>
<th>DJD</th>
<th>n</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate/Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>312</td>
<td>3.8 ± 0.9 b</td>
<td>4.3 ± 0.9 a</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>OI</td>
<td>292</td>
<td>0.27 ± 0.06 b</td>
<td>0.31 ± 0.06 ac</td>
<td>0.26 ± 0.07 b</td>
</tr>
<tr>
<td>SI</td>
<td>128</td>
<td>0.25 ± 0.14 b</td>
<td>0.42 ± 0.29 a</td>
<td>0.32 ± 0.17</td>
</tr>
</tbody>
</table>

BW=body weight, OI=obesity index, SI=subluxation index

a Significantly different from the normal group.
b Significantly different from the mild group.
c Significantly different from the moderate/severe group.
Table 2-4 Norberg angle (NA) among the groups based on the radiographic grades.

<table>
<thead>
<tr>
<th>NAº (1)</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate/Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=312</td>
<td>100.7 ± 7.0 bc</td>
<td>93.8 ± 8.7 a</td>
<td>88.3 ± 8.4 a</td>
</tr>
<tr>
<td>NAº (2)</td>
<td>100.9 ± 6.9 b</td>
<td>96.0 ± 7.3 a</td>
<td>96.7 ± 1.5</td>
</tr>
<tr>
<td>n=290</td>
<td>94.0 ± 3.1 bc</td>
<td>82.3 ± 6.4 a</td>
<td>85.2 ± 7.7 a</td>
</tr>
<tr>
<td>NAº (3)</td>
<td>22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA (1)=all cats, NA (2)=cats without HD, NA (3)=cats with HD.

a Significantly different from the normal group.
b Significantly different from the mild group.
c Significantly different from the moderate/severe group.

Table 2-5 Frequency of gross lesions between breeds, genders, and the age-related groups.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Normal</th>
<th>DJD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic</td>
<td>91 (65.0)</td>
<td>49 (35.0)</td>
</tr>
<tr>
<td>Purebred</td>
<td>10 (62.5)</td>
<td>6 (37.5)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52 (66.7)</td>
<td>26 (33.3)</td>
</tr>
<tr>
<td>Intact*</td>
<td>37 (77.1)</td>
<td>11 (22.9)</td>
</tr>
<tr>
<td>Castrated*</td>
<td>15 (50.0)</td>
<td>15 (50.0)</td>
</tr>
<tr>
<td>Female</td>
<td>49 (62.8)</td>
<td>29 (37.2)</td>
</tr>
<tr>
<td>Age*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>28 (84.8)</td>
<td>5 (15.2)</td>
</tr>
<tr>
<td>Adult</td>
<td>73 (59.3)</td>
<td>50 (40.7)</td>
</tr>
</tbody>
</table>

( ) indicates percentage.
* Significantly different at p<.05.

Table 2-6 Body weight, obesity index, and subluxation index among the groups based on the gross pathologic grades.

<table>
<thead>
<tr>
<th>DJD</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate/Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW=body weight, OI=obesity index, SI=subluxation index.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=312</td>
<td>3.9 ± 0.9</td>
<td>3.9 ± 1.1</td>
<td>3.7 ± 1.0</td>
</tr>
<tr>
<td>OI=292</td>
<td>0.27 ± 0.05</td>
<td>0.28 ± 0.07</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>SI=128</td>
<td>0.27 ± 0.15</td>
<td>0.28 ± 0.19</td>
<td>0.21 ± 0.12</td>
</tr>
</tbody>
</table>
Table 2-7 Norberg angle (NA) among the groups based on the gross pathologic grades.

<table>
<thead>
<tr>
<th>NA&lt;sup&gt;a&lt;/sup&gt; (1)</th>
<th>312</th>
<th>99.7 ± 7.0</th>
<th>98.9 ± 10.0</th>
<th>100.3 ± 8.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA&lt;sup&gt;a&lt;/sup&gt; (2)</td>
<td>290</td>
<td>100.9 ± 6.9</td>
<td>96.0 ± 7.3</td>
<td>96.7 ± 1.5</td>
</tr>
<tr>
<td>NA&lt;sup&gt;a&lt;/sup&gt; (3)</td>
<td>22</td>
<td>90.8 ± 6.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.0 ± 7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.8 ± 5.8</td>
</tr>
</tbody>
</table>

NA<sup>a</sup> (1)=all cats, NA<sup>a</sup> (2)=cats without HD, NA<sup>a</sup> (3)=cats with HD.
<sup>a</sup> Significantly different from the normal group.
<sup>b</sup> Significantly different from the mild group.
<sup>c</sup> Significantly different from the moderate/severe group.

Table 2-8 Correlation between radiographic and gross pathologic grades.

<table>
<thead>
<tr>
<th>Gross pathologic grades</th>
<th>Normal</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>96 (212)</td>
<td>18 (34)</td>
<td>20 (29)</td>
<td>0 (1)</td>
<td>134 (276)</td>
</tr>
<tr>
<td>Radiographic grades</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>3 (12)</td>
<td>9 (11)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>14 (25)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>3 (5)</td>
<td>0 (0)</td>
<td>6 (8)</td>
</tr>
<tr>
<td>Severe</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>101 (227)</td>
<td>29 (47)</td>
<td>25 (36)</td>
<td>1 (2)</td>
<td>156 (312)</td>
</tr>
</tbody>
</table>

( ) indicates a number of joints.
Figure 2-1 Ventrodorsal radiographs of hip joints.

(A) A hip joint with a flattened dorsocranial acetabular rim.

(B) A hip joint with a laterally pointed dorsocranial acetabular rim.

(C) A hip joint with mild degenerative changes. The contour of the dorsocranial acetabular rim is indistinct, suggesting a new bone formation. Acetabular fossa appears slightly sclerotic.

(D) A hip joint with severe degenerative changes. A large osteophyte is apparent on the dorsocranial acetabular rim. There is a sclerotic change in the acetabular fossa. Subluxation of the femoral head is apparent most likely due to hip dysplasia.
Figure 2-2 Photographs of a normal left femoral head.

(A) A ventrodorsal radiograph of the femoral head.

(B) A gross photograph of the femoral head. Normal femoral cartilage has a smooth and glistening layer of articular cartilage. An arrow indicates the cranial aspect.

(C) A photograph of a thin-section specimen. Articular cartilage is thick around the attachment of the round ligament. Thickened subchondral bone is also evident around the round ligament. An arrow indicates the cranial aspect.

(D) A high-detail radiograph of the same specimen as in (C). Subchondral bone is thick around the round ligament. Thickened subchondral bone is also seen on the caudal portion of the femoral head where a small ridge is present.
Figure 2-3 Photomicrographs of articular cartilage of a normal femoral head (same femoral head as in Figure 2-2).

(A) The cranial portion of the femoral cartilage. A characteristic zonal appearance of hyaline cartilage is seen. (H & E stain, ×100)

(B) The cranial aspect of the femoral cartilage. Intense stainability of the transitional zone against Safranin-O demonstrates a high concentration of proteoglycans. Pericellular areas in the deep zone are more intensely stained. (Safranin-O stain, ×100)

(C) The caudal aspect of the femoral cartilage. Hyaline cartilage is gradually replaced with fibrous cartilage. Slight surface roughening is seen at this portion. A zonal pattern characteristic of hyaline cartilage is not seen. (H & E stain, ×100)

(D) The caudal aspect of the femoral cartilage. Diminished stainability on the Safranin-O is noted at the portion where fibrous cartilage replaces hyaline cartilage. (Safranin-O stain, ×100)
Figure 2-4 Photographs of a normal left acetabulum.

(A) A ventrodorsal radiograph of the acetabulum.

(B) A gross photograph of the caudal segment of the acetabulum. Note a smooth and glistening appearance of normal acetabular cartilage.

(C) A photograph of a thin-section specimen. An arrow indicates the dorsal aspect.

(D) A high-detail radiograph of the same specimen as in (C).
Figure 2-5 Photomicrographs of a normal dorsocaudal acetabular rim. (same acetabulum as in Figure 2-4).

(A) The dorsal periphery of the cartilage is partly composed by fibrous cartilage. A relatively large acetabular labrum attaching to the acetabular rim is seen. (H & E, ×40)

(B) A low stainability of the fibrous cartilage at the dorsal periphery of the rim is noted. (Safranin-O, ×40)
Figure 2-6 Photographs of a left acetabulum with surface irregularity on the caudal aspect.

(A) A ventrodorsal radiograph of the acetabulum. Degenerative changes are not seen in the hip joint.

(B) A gross photograph of the caudal segment of the acetabulum. Surface dullness is noted on the dorsal periphery of the cartilage (arrow). Mildly thickened synovial membrane is also present.

(C) A photograph of a thin-section specimen.

(D) A high-detail radiograph of the same specimen as in (C). Small osteophytes are noted on the ventral portion of the dorsal acetabular rim (arrow) and the ventral acetabular rim (arrow head).
Figure 2-7 Photomicrographs of the dorsocaudal acetabular rim with mild gross lesion (same acetabulum as in Figure 2-6).

(A) Surface roughening of the dorsal periphery of the cartilage is seen. (H & E, ×40)

(B) Roughened cartilage shows low stainability. The area with diminished stainability extends ventrally (arrow). (Safranin-O stain, ×40)
Figure 2-8 Photographs of a left femoral head and acetabulum with radiographically severe DJD and grossly moderate lesions.

(A) A ventrodorsal radiograph. A large osteophyte is seen on the dorsocranial acetabular rim. Subluxation of the femoral head is also evident.

(B) A gross photograph of the medial aspect of the femoral head. Generalized surface dullness is noted. An arrow indicates the cranial aspect.

(C) A gross photograph of the caudal aspect of the femoral head. At the region close to the lateral margin of the cartilage an area of worn cartilage is seen (arrow head). This is a partial loss of cartilage (moderate lesion).

(D) A gross photograph of the caudal segment of the acetabulum. Articular cartilage also shows generalized dullness.
Figure 2-9 Photographs of the thin-section specimens and high-detail radiographs of the femoral head and acetabulum of a cat with radiographically severe DJD (same femoral head and acetabulum as in Figure 2-8).

(A) A photograph of a thin-section specimen. Subchondral bone appears thickened at the caudal aspect (arrow heads). An arrow indicates the cranial aspect.

(B) A high-detail radiograph of the same specimen as in (A). Thickened subchondral bone is noted at the caudal aspect (arrow heads).

(C) A photograph of a thin-section specimen. A synovial membrane is thickened. An arrow indicates the dorsal aspect.

(D) A high-detail radiograph of the same specimen as in (C).
Figure 2-10 Photomicrographs of articular cartilage from a cat with radiographically severe DJD (same joint as in Figure 2-8).

(A) Articular cartilage of the cranial femoral head. Hypocellularity, low stainability, and roughening of the articular surface are noted. (H & E, ×100)

(B) Articular cartilage of the cranial femoral head. Diminished stainability in the upper part of the transitional zone suggests a loss of proteoglycans. (Safranin-O, ×100)

(C) Articular cartilage of the dorsocaudal acetabular rim. A small osteophyte (articular lipping) is present. The periphery of the rim is composed mainly of fibrous cartilage. (H & E, ×40)

(D) Articular cartilage of the dorsocaudal acetabular rim. A region with low stainability is present and extends ventrally (arrow). (Safranin-O, ×40)
CHAPTER 3
MAGNETIC RESONANCE IMAGING OF FELINE HIP JOINTS

Introduction

Degenerative joint disease (DJD) is characterized by progressive degradation of articular cartilage followed by changes in underlying bones and surrounding soft tissues (Johnston 1997). In people, van Saase et al. (1989) reported that DJD was the most common joint disease, especially among elderly people. In dogs, DJD was commonly associated with developmental joint disorders such as elbow dysplasia and hip dysplasia (Smith et al., 1995; Keller et al., 1997). Recently, DJD was also described in cats as a common disease, especially among aged cats (Hardie et al., 2002; Kamishina and Miyabayashi 2002).

Diagnostic techniques of DJD have been developed with a special emphasis on early detection of cartilage lesions, because a degenerative process in articular cartilage is progressive and irreversible in nature (Mankin 1974).

In people, magnetic resonance imaging (MRI) is presently the most desirable non-invasive imaging modality in evaluating articular cartilage. Numerous studies have been conducted to establish cartilage specific sequences.

Spin echo (SE) sequences with various weighting have been extensively studied for articular cartilage imaging. In an early study by Lehner et al. (1989), a single homogeneous layer with high signal intensity was observed in normal bovine patellar cartilage on T1-weighted SE images. However, on T2-weighted SE images, high signal intensity of a superficial layer was differentiated from low signal intensity of a deep
The water content in cartilage that decreased from the superficial layer (82%) to the deep layer (76%) was thought to influence T1 and T2 relaxation times and to contribute a zonal pattern of cartilage on the T2-weighted SE images.

Modl et al. (1991) reported that on T1-weighted and T2-weighted SE images, a zonal appearance of articular cartilage on MRI correlated with histological zones of human normal articular cartilage. In the study, cadaveric knees and ankles were imaged with a 1.5-tesla (T) MRI unit. On T1-weighted SE images, a hypointense superficial layer, an intermediate-signal-intense middle layer, and a hypointense deep layer were observed. On T2-weighted SE images, the middle layer appeared hyperintense. On the MR images, the superficial layer, middle layer, and deep layer occupied an average of 16% (range; 7-45%), 31% (range; 10-75%), and 53% (range; 17-80%) of the total cartilage thickness, respectively. On the histological sections, a superficial zone, transitional zone, and two deep zones (radial zone and calcified cartilage) occupied an average of 5% (range; 3-12%), 42% (range; 22-68%), and 53% (range; 27-72%) of the total cartilage thickness, respectively. The three layers observed on the MR images corresponded in location, but not exactly in thickness.

Rubenstein et al. (1993) observed a laminated structure of normal bovine patellar cartilage with T1-weighted, T2-weighted, and proton-density SE sequences. In the study, a 1.5-T MRI unit was used and the effects of collagen orientation on a laminated appearance of cartilage were investigated. With all sequences, a hyperintense superficial layer, hypointense transitional layer, and intermediate intensity of a deep layer were observed. A distinct hypointense fourth layer was proved to represent the calcified cartilage and a subchondral bone on histological sections. Cartilage specimens were
imaged with specimen rotation about the vertical axis in 5° increments between +75° and -130°. The results showed that the laminated appearance of cartilage was largely dependent on the orientation of the cartilage with respect to the main magnetic induction field (B0). The trilaminar appearance of the cartilage was most evident when the surface of the cartilage faced 0° (perpendicular) and -90° (parallel) to the B0. When the patellar surface oriented 55° to the B0, the cartilage appeared homogeneous. The signal intensity of the second (transitional) layer dramatically increased with rotation (the peaks at +55° and -55°) and contributed to the laminated appearance of cartilage. Zonal differences of the collagen orientation were confirmed on electron microscopy and believed to affect the laminated appearance of the cartilage.

Image resolution and an echo time (TE) were also reported to affect laminated appearance of cartilage. In a study by Rubenstein et al. (1996), excised normal bovine patellae were used to make a cylindrical cartilage-bone specimen. Two articular surfaces of the specimens were matched and placed in a 55-mm birdcage coil and imaged with T1-weighted SE with various TE (5.5, 10, 20, and 40 msec) on a 1.9-T MRI unit. The images obtained with a TE of 5.5 msec showed a uniform hyperintense layer of articular cartilage with an ill-defined hypointense line at the interface between the two cartilage surfaces. On the images obtained with TE of 10 and 20 msec, the hypointense line between the two cartilage surfaces became more distinct and an intermediate-signal-intensity layer was seen, extending through the deep half of the cartilage. On the images with TE of 40 msec, the hypointense line at the cartilage interface appeared thicker. A hyperintense middle layer was seen between the hypointense superficial line and the intermediate-signal-intensity of the deep layer. To determine the effects of image
resolution, T1-weighted SE images were made on a 1.5-T MRI unit, using a 256 x 256 matrix, 8 cm field of view (312 µm in-plane resolution), two acquisitions, and 3 mm section thickness with a 1.5 mm intersection gap. The images were compared with those obtained by using a 512 x 512 matrix (156 µm in-plane resolution) while maintaining other imaging parameters. On the images with lower image resolution (312 µm in-plane resolution), an indistinct hypointense line between the two cartilage specimens were noted, while the line was clearly seen on the images with higher image resolution (156 µm in-plane resolution).

Accuracies of T1 and T2-weighted SE images in detecting cartilage lesions have been reported. In 20 human cadaveric knees, Hodler et al. (1992) reported that T1-weighted, T2-weighted, and proton-density weighted sequences on a 1.5-T MRI unit were not sufficient in detecting cartilage lesions in human knees. In the study, on anatomical sections 82 lesions were identified. In an unblinded fashion, 72% (59 lesions), 68.3% (56 lesions), and 60% (49 lesions) of the lesions were detectable on the T1-weighted, T2-weighted, and proton-density SE images, respectively. Subsequently, images of a subset of 35 pathologic and 35 normal cartilage surfaces were blindly evaluated with each sequence. Twenty-five of the 35 lesions and 24 of 35 normal cartilage surfaces were correctly diagnosed on the simultaneous analyses of the T1-weighted, T2-weighted, and proton-density SE images. The sensitivity, specificity, and accuracy were 71.4%, 68.6%, and 70.0%, respectively.

Accuracies of T1-weighted, T2-weighted, and proton-density SE sequences were also reported by Recht et al. (1993). In the study, 10 cadaveric knees were evaluated with a 1.5-T MRI unit. A total of 44 regions were evaluated and 25 had macroscopic
cartilage lesions. On the MR images, the sensitivity of T1-weighted, T2-weighted, and proton-density SE were 52%, 48%, and 28%, respectively. The specificity and accuracy were 95% and 70%, 58% and 52%, and 79% and 50%, respectively.

More recently, fat-suppressed three-dimensional spoiled gradient-echo sequence (FS 3-D SPGR) and fat-suppressed fast spin-echo sequence (FS FSE) have been proposed as the two best sequences for the articular cartilage imaging (Recht et al., 1993; Recht and Resnick 1994).

On FS 3-D SPGR images, excellent contrast between cartilage and surrounding structures was achieved. Articular cartilage appeared as a bright structure (high signal intensity) compared to dark surrounding tissues such as synovial fluid, bones, fat, and muscles (low signal intensity) (Chandnani et al., 1991; Recht et al., 1996). Contrast to noise ratios were compared among FS 3-D SPGR with various TEs and flip angles (Recht et al., 1993). The contrast to noise ratios for cartilage versus joint fluid and cartilage versus bone marrow were calculated as the signal intensity of cartilage minus the signal intensity of joint fluid (or bone marrow) divided by the standard deviation of noise. The TE of 5 with the flip angle of 30°, and TE of 10 with the flip angle of 60° showed the highest contrast to noise ratios. However, comparison of contrast to noise ratios among FS 3-D SPGR and other common sequences were not performed.

The volume (3-D) acquisition allows a very thin slice to be obtained, resulting in a reduction of partial volume artifacts, thereby high-resolution images (Yao et al., 1992). Another advantage of FS 3-D SPGR was that of improved signal-to-noise ratio for a given slice thickness compared to those obtained by SE sequences (Recht et al., 1996).
Because of these advantages a high accuracy of this sequence in detecting cartilage lesions has been reported. Disler et al. (1996) evaluated the accuracy of this sequence and compared to those of T1-wighted SE, T2-weighted dual-spin echo, and gradient-echo sequences in 47 patients who underwent MRI and subsequent arthroscopy. Six articular surfaces were evaluated in each knee; therefore a total of 282 articular surfaces were evaluated. In 32 patients, 79 cartilage lesions were found. The sensitivity of FS 3-D SPGR evaluated by two readers was significantly higher (75-85%) than that of other sequences (29-38%). However, no difference in specificity was detected (97% versus 97%).

Trattnig et al. (2001) reported that the bilaminar pattern of articular cartilage in a normal tibial condyle in human on FS 3-D SPGR MR images was correlated with two histological zones, that is proteoglycan-rich and proteoglycan-free zones. In the study, the bilaminar pattern changed to a trilaminar pattern in an aged group (>30 years old). The authors proposed that this change in a laminar pattern could be attributed to a reduction of proteoglycan contents from the deepest zone that led to the increased signal intensity, resulting in a trilaminar appearance of tibial condylar cartilage.

Other investigators however have questioned the laminar patterns of articular cartilage on SPGR images. When a short echo time (TE) was used to obtain a 3-D SPGR MR image, a false laminar appearance of cartilage was observed, resulting from a truncation artifact (Erickson et al., 1996; Frank et al., 1997). The artifact resulted from insufficient sampling of a structure with high spatial frequencies, which associated with the thickness of the cartilage and the pixel dimension chosen (Trattnig et al., 2001).
Another drawback of the FS 3-D SPGR sequence was its relatively long imaging time compared to other commonly used sequences.

Fat-suppressed fast spin-echo sequences significantly decreased a total image time, thereby improved the signal-to-noise ratio. T2-weighted FS FSE sequences have been used for cartilage imaging since a high contrast between the cartilage (intermediate signal intensity) and joint fluid (high signal intensity) can be obtained. The high contrast resulted from T2 weighting and Magnetization transfer (MT) effects that occur in tissues with a high concentration of macromolecules (Wolff et al., 1991). Magnetization transfer effects have been known to decrease the signal from articular cartilage, but minimally affect the signal from joint fluid, resulting in a high contrast at a cartilage-fluid interface (Wolff et al., 1991; Gray et al., 1995).

Bredella et al. (1999) reported the accuracy of T2-weighted FS FSE images in evaluating cartilage lesions in human knees. In the study, 780 articular surfaces in 130 patients who underwent arthroscopy were evaluated. A normal articular cartilage appeared intermediate signal intensity that was easily differentiated from a high signal intensity of synovial fluid and a low signal intensity of the subchondral bone. The T2-weighted FS FSE sequence was particularly sensitive to detect the early lesions. The early lesions, softening of articular cartilage confirmed by arthroscopy, appeared as foci with increased signal intensity within cartilage on the T2-weighted FS FSE images. Surface irregularity with increased signal intensity of cartilage corresponded to the lesions with shallow defects on arthroscopy. Increased signal intensity was also noted in the subchondral bone. This finding was interpreted as bone marrow edema. The best
results were obtained by a combination of the axial and coronal images; the sensitivity, specificity, and accuracy were reported to be 93%, 99%, and 98%, respectively.

The accuracy of a proton-density FS FSE sequence in detecting cartilage lesions was reported by Potter et al. (1998). A total of 616 surfaces of articular cartilage in 88 human knees were evaluated and the findings were compared with those of arthroscopy. Normal articular cartilage appeared uniform thickness with homogeneous intermediate signal intensity that contrasted with the low signal intensity of a subchondral bone and the high signal intensity of joint fluid. Early lesions on patellar cartilage were noted on the MR images as focal blister on the articular surface accompanied by a lack of sharp interface between the articular surface and joint fluid. Hyperintensity of the signal in the superficial layer was also noted with early lesions. These lesions were evaluated as the earliest lesions on arthroscopy. More advanced lesions such as partial-thickness and full-thickness defects of articular cartilage were readily noted on MR images, owing to the high contrast between cartilage and joint fluid. The sensitivity, specificity, and accuracy were 87%, 94%, and 92%, respectively. The authors suggested that the proton-density FS FSE sequence was accurate to especially detect morphological abnormalities rather than changes in signal intensity. In addition, the high contrast between articular cartilage and surrounding tissues was achieved with this sequence even in the absence of joint fluid.

Another advantage of MRI is its ability to provide biochemical information of articular cartilage. It has been well known that biochemical changes of articular cartilage composition occur in early stages of DJD. One of the most important abnormalities seen in early stages of DJD is a loss of glycosaminoglycans (GAGs), a constitutive part of
proteoglycans, from the extracellular matrix of the cartilage (Malemud 1991).

Glycosaminoglycans contain abundant negatively charged side groups that confer a negative charge density to the cartilage matrix. The negatively charged side groups are “fixed” to the matrix and therefore the degree of the charge is referred to as fixed charge density (FCD). Several MRI techniques have been developed to detect and monitor the changes in proteoglycan contents in articular cartilage by estimating the FCD of cartilage.

Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) was validated in in vitro studies (Bashir et al., 1996, 1999; Allen et al., 1999) and an in vivo study applying this technique to human patellar cartilage (Bashir et al., 1997). The technique is based on a distribution of a negatively charged paramagnetic contrast agent, gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA2-), into the cartilage matrix where GAGs are depleted (Bashir et al., 1996). Since Gd-DTPA2- has a concentration-dependent effect to drop T1-relaxation times, FCD in the tissue (GAGs contents) can be estimated from the Gd-DTPA2- concentration by measuring the T1 relaxation time in cartilage (Stanisz and Henkelman 2000). The problem of this method was that full penetration of Gd-DTPA2- into the cartilage matrix required several hours of exercise. In addition, after administration of Gd-DTPA2- the concentration of Gd-DTPA2- changes with time during the imaging, therefore the images had to be acquired quickly to ensure consistent interpretation of the results.

Sodium MR imaging is another nondestructive technique for the evaluation of the GAG contents in articular cartilage. Sodium MRI has been validated in recent in vitro studies using bovine patellar cartilage (Insko et al., 1999; Regatte et al., 1999; Borthakur et al., 2000; Shapiro et al., 2000, 2002). The technique was also reported in in vivo
studies using human patellar cartilage (Reddy et al., 1998; Shapiro et al., 2002) and intervertebral disks (Insko et al., 2002).

Shapiro et al. (2002) compared the FCD measurements obtained by sodium MRI and those calculated by the standard dimethylmethylene blue assays, using enzymatically degraded bovine patellar cartilage. Two measurements strongly correlated each other ($r^2 = 0.81$). On the sodium map calculated images, one side of patellar cartilage that was degraded by trypsin consistently showed lower sodium contents (average sodium; 261mM) than the other control side (average sodium; 316mM), corresponding to the FCD of –192mM and –260mM, respectively.

Quantification of sodium contents in the human wrist was reported (Borthakur et al., 2002). Six wrist joints from healthy volunteers were imaged with sodium and proton MRI, using a 4-T MRI unit. Sodium phantoms were made with different concentrations of sodium (100, 150, 200, 250mM/L) in 10% wt/vol agarose gel as described previously (Shapiro et al., 2000). The phantoms were simultaneously imaged to calculate the sodium contents in the wrists. Sodium map calculated images clearly showed articular cartilage surrounding the major bones in the wrist. A significantly higher sodium concentration was detected in cartilage (200 mM/L) than in the surrounding ligaments and synovial fluid (115-140 mM/L).

Borthakur et al. (2000) reported the sensitivity of sodium MRI in detecting a proteoglycan loss. Enzymatically degraded bovine patellar cartilage was imaged with sodium and proton MRI. Decreased sodium signal intensity in the region of the degraded cartilage was clearly observed on the sodium MR images, while the change was not consistent on the proton MR images. The changes in signal intensity on the sodium MRI
correlated with the degree of proteoglycan loss confirmed by a spectrophotometric assay, while T1 and T2 profiles on the proton MRI did not correlated with the proteoglycan loss.

Although sodium MRI has been used to correlate a sodium concentration and proteoglycan contents of cartilage, no study has been conducted to investigate a correlation of a sodium concentration and sulfation patterns of proteoglycans. A proportion of chondroitin sulfate (CS)-4 and CS-6 has been known to change with aging and DJD. In dogs, Harab and Mourao (1989) reported that CS-4 contents in proximal tibial cartilage decreased with aging while CS-6 contents remained constant. They also found depth-dependent changes in the contents of CS-4 and CS-6. The CS-4 contents markedly increased from the articular surface to the deeper region while the contents of CS-6 remained constant. Similar age-related and depth-dependent variations of chondroitin sulfation patterns were observed in human articular cartilage, but Bayliss et al. (1999) added that the variations were also dependent on the topographic locations on the joint surface. In equine articular cartilage, Brown et al. (1998) reported the ratio of CS-4 and CS-6 in normal cartilage and osteoarthritic cartilage. The ratio of CS-6 to CS-4 increased from birth until 2 years old and then decreased in old horses (> 10 years). In osteoarthritic cartilage, the ratio decreased from that of a normal age-matched group. Since the proportion of CS-4 and CS-6 is an important indicator of aged and degenerative cartilage, a non-invasive technique to detect a change of sulfation patterns should be useful.

The first objective of the present study was to describe a MRI appearance of feline hip joints, using a high-magnetic field (4.7-T) MRI unit. The second objective was to
assess the feasibility of sodium MRI of feline hip joints and to investigate a correlation between sodium concentrations and a sulfation pattern of articular cartilage.

Material and Methods

Radiographic and Gross Pathologic Evaluations

Twelve cat cadavers were collected from a local animal shelter immediately after euthanasia. There were 7 males and 5 females with a mean ± SD body weight of 3.5 ± 1.0 kg. All cats were skeletally mature domestic cats. The cats were placed in a trough in dorsal recumbency. Ventrodorsal radiographs of the hip joints with hind limbs adducted and extended parallel to the vertebral column were made in all cats, using a clinical radiographic machine (Innovet, Summit industries Inc, Chicago, IL). Hip joints were graded based on the presence and severity of radiographic signs of DJD. The grading scheme was as follows; normal (no osteophyte), mild DJD (osteophyte <1mm), moderate DJD (osteophyte <2mm), and severe DJD (osteophyte >2mm).

After MRI, all articular cartilage of the femoral heads and acetabula were grossly examined under a stereo microscope and graded for degenerative changes. The gross pathologic grading scheme was as follows; normal (intact articular cartilage), mild DJD (surface dullness or irregularity), moderate DJD (surface irregularity and/or a partial loss of cartilage), and severe DJD (a complete loss of cartilage and subchondral bone exposure). Locations of the lesions were recorded as dorsal, cranial, and caudal aspects in relation to the portion of the round ligament attachment.

High-detail Radiography

All left femoral heads and acetabula were serially sectioned transversely at 2 mm intervals, using a low speed saw (Isomet 11-1180, Buehler Ltd, Evanston, IL) with a diamond-coated blade (Diamond Wafering Blade 11-4245, Buehler Ltd, Evanston, IL).
High detail radiographs of the thin-section specimens were made, using a cabinet radiographic machine (Faxitron Model MX-20, Faxitron X-Ray Corporation, Wheeling, IL) with non-screen films (Kodak X-Omat TL, Eastman Kodak Company, Rochester, NY). The following technique was used for all specimens: 30 kVp, 0.3mA, 60sec, at 57.2 cm FFD. High-detail radiographs were reviewed for the presence of an osteophyte formation, enthesophyte formation, and subchondral bone sclerosis. The specimens were placed in a neutral-buffered 10% formalin solution for the histopathologic examination.

**Histopathologic Evaluation**

Histopathologic examinations were performed in all left femoral heads and left acetabula. The thin-section specimens were decalcified in a neutral-buffered 25% formic acid solution under gentle agitation. Duration of the decalcification process was determined based on the high detail radiographs. After this process, the specimens were rinsed in running tap water, dehydrated in a graded series of ethanol, and embedded in paraffin. Embedded bones were sectioned in 5 microns and stained with H & E and Safranin-O stain.

**Analyses of Chondroitin Sulfates**

The amount and the sulfation pattern of chondroitin sulfates were evaluated. This was performed on all right femoral cartilage after the gross examinations. Femoral articular cartilage was removed and diced under a laminar flow cabinet. After washing the cartilage with a Hank’s balanced salt solution, the cartilage was placed in 10 ml of a 4M guanidine hydrochloride solution containing 50mM Tris-HCl, 10mM N-ethylmaleimid, 0.36 mM pepstatin A, and 1mM phenylmethysulfonyl fluoride (PMSF) at 4°C for overnight (Oegema et al., 1979). The extracted solution was strained through a 70-µm filter (Cell Strainer, Becton Dickinson Labware, Franklin Lakes, NJ). To
eliminate other proteins, samples were subjected to the equilibrium density centrifugation. Cesium chloride (Fisher Scientific, Pittsburg, PA) was added to the samples at a concentration of 0.55 g/g of the sample solution. The samples were centrifuged for 48 hours at 40,000 rpm, 8°C, using a fixed angle rotor (Type T70.1, Beckman Instrument, Inc., Palo Alto, CA) in a centrifuge tube (Ultra-Clear Centrifuge tube 344322, Beckman Instrument, Inc., Palo Alto, CA). Newly synthesized proteoglycans were collected from the deepest layer in the tube (Maldonado and Oegema 1992). The collected samples were run into a PD-10 column. Then elution was performed with a 0.02M Tris-HCl solution. Obtained samples were stored in -20°C until following digestion.

The extracted samples (275µl) were digested with 5mU of chondroitinase ABC (Seikagaku America, Inc., Ijamsville, MD) containing 80 µl of 100mM Tris-HCl (Sigma Chemical Co. Ltd., St.Luis, MO), and 40µl of 0.27mM cinamic acid (CA, Sigma Chemical Co. Ltd., St.Luis, MO) for 3 hours at 37°C. After 3 hours of digestion, digestion was terminated by placing the samples in boiling water for 1 minute (Karamanos et al., 1995). Capillary electrophoresis (BioFocus®3000, Bio-Rad, Hercules, CA, U.S.A) was carried out to quantify the amount and the proportion of CS-4 and CS-6 in the digested samples. A fused silica capillary column (50mm i.d., 375mm o.d., 50cm length, Bio-Rad, Hercules, CA, U.S.A) was used. The digested samples were loaded under vacuum and electrophoresed for 15 minutes at 23°C, 15kV in a 40mM phosphate solution (Sigma Chemical Co. Ltd., St.Luis, MO), containing 40mM lauryl sulfate (Sigma Chemical Co. Ltd., St.Luis, MO) and 10mM sodium borate (Sigma Chemical Co. Ltd., St.Luis, MO) at pH 9.0. The eluant was monitored at 232nm (Carney
and Osbone, 1991). Peak areas for both CS-4 and CS-6 were standardized by that of the standard marker (CA). Absolute amounts of CS-4 and CS-6 were calculated from the standard curve which had been established by measuring known concentrations of serially diluted disaccharide samples (Seikagaku America, Inc., Ijamsville, MD) as described by Maeda et al. (Maeda et al., 2001). The electrophoresis was repeated three times.

In order to estimate the difference in the proportion of CS-6 to CS-4 between normal cartilage and degenerative cartilage determined by the gross pathologic grades, the obtained proportions were compared by Student t-test with the p value set at 0.05 (SAS Version 6.12).

**Computed Tomography**

In 3 cats, computed tomography (CT) was performed on the hip joints, using a Tomoscan M-EG (Philips medical systems) after radiographic examinations. The cats were placed in a trough in dorsal recumbency. A sponge was placed between the knees and the knees were taped so that the hind limbs adducted and extended parallel to each other. The used imaging parameters were as follow, 130-140 kVp, 30-40 mA, field of view of 60-82 mm, and the matrix size of 512 x 512. Contiguous transverse 2.0 mm slices were obtained.

**Magnetic Resonance Imaging**

**Proton-MR imaging**

Magnetic resonance imaging was performed in all cats. Magnetic resonance imaging was performed with a 4.7-T Oxford magnet with a Bruker Console, using a custom-built transmit-receive quadrature driven birdcage coil with an internal diameter of 11.2 cm. The coil was tuned to 200.18 MHz which is the resonance frequency of 1H at
4.7-T. T2-weighted fat-suppressed (FS) Fast Spin Echo (FSE) images were acquired with the repetition time (TR) of 3687.5 msec, echo time (TE) of 25 msec, and rare factor of 8, resulting in an effective TE of 112.5 msec. Proton-density FS FSE images were acquired with the TR of 3531.5 msec, TE of 9 msec, rare factor of 4, resulting in an effective TE of 22.5 msec. Eight acquisitions were used for the T2-weighted FS FSE and four acquisitions were used for the proton-density FS FSE. Frequency-selective fat presaturations were used. For both sequences, section thickness was 1.7 mm with 0.17 mm interspaces, field of view was 90 mm, and the matrix was 384 x 384, yielding an in-plane resolution of 234 µm². The total imaging time was 23 min 36 sec for the T2-weighted FS FSE, and 16 min 57 sec for the proton-density FS FSE. A standard marker, a 1 ml syringe containing 0.9% sodium chloride solution, was placed on the cats.

In order to compare image contrast between the proton-density FS FSE images and the T2-weighted FS FSE images, a signal to noise ratio (SNR) of femoral cartilage, joint fluid, and bone marrow were measured. The SNR was measured as the signal intensity of a tissue divided by the standard deviation of noise. The signal intensity of air was used to estimate noise. The SNRs were compared between two sequences, using Student t-test (SAS Version 6.12). Statistical significance was set at p<0.05.

**Sodium-MR imaging**

Sodium imaging was performed on one cat with radiographically normal hip joints. The imaging parameters were as follows; 2-D gradient-echo pulse sequence, TR/TE of 60/2.5 msec, slice thickness of 8.0mm with no intersection gap, the matrix of 128 x 128, field of view of 16.0mm, and 32 acquisitions. A total imaging time was 4min 5sec.

For sodium phantom imaging, 4 sodium phantoms were made, using 1 ml syringes. Four 1ml-syringes contained 1M, 500mM, 250mM, and 125mM/L sodium chloride
solutions, respectively. Sodium MRI was performed on the same 4.7-T magnet with a custom-built birdcage coil (internal diameter of 11.2 cm) tuned to 52.95 MHz which is the resonance frequency of 23Na at 4.7-T. A 3-D gradient-echo pulse sequence with the TR of 100 msec and the TE of 2.7 msec was used. Section thickness was 40.0 mm with no intersection gap. The matrix was 128 x 64 x 16. Field of view was 12.0 x 12.0 x 4.0 mm. Eight acquisitions were made. A total imaging time was 13min 39sec. Signal intensity was measured by placing a region of interest on each phantom. Obtained values were then plotted against the sodium concentrations to make a calibration curve for the sodium phantoms. To evaluate a correlation between the signal intensity and the sodium concentration, Pearson’s r-value was calculated (JMP in version 3.2.1).

Results

Radiographic and Gross Pathologic Findings

Based on the radiographic examinations, 11 cats had normal hip joints and 1 cat had mild hip DJD. The cat with hip DJD showed small osteophytes on the dorsocranial acetabular rims and a mild sclerotic acetabular fossa (Fig 3-2-A).

On the gross examinations, there were 9 cats with normal articular cartilage on the femoral heads and acetabula. There were 3 cats with gross abnormalities on articular cartilage of the femoral heads and acetabula or both. Of those 3 cats, 1 had mild gross lesions on the dorsal aspects of the both femoral heads, and a mild gross lesion on the dorsal aspect of the left acetabulum. The second cat had a moderate lesion on the caudal aspect of the right femoral head. The last cat had a moderate lesion on the caudal aspect of the right acetabulum.
High-detail Radiographic Findings

On the high-detail radiographs, radiographically and grossly normal femoral heads showed uniform thickness of a subchondral bone (Figure 3-1-C). A trabecular bone appeared dense under the region where the round ligament attached to the femoral head. A similar dense trabecular pattern was noted at the caudal aspect of the femoral head. A cortical bone of the femoral neck was thicker on the cranial aspect. Thickness of the subchondral bone of acetabula was difficult to evaluate due to concavity of acetabula.

Femoral heads with mild gross lesions showed a thickened subchondral bone (Figure 3-2-E). However, a new bone formation was not observed. In the acetabula with mild gross lesions, an apparent change was not detected.

Histopathologic Findings

Radiographically and grossly normal articular cartilage of the femoral heads and acetabula showed a characteristic pattern of hyaline cartilage on the H & E stain (Figure 3-1-D). In a superficial zone, chondrocytes that lined parallel to the articular surface were noted. Underneath the superficial zone where the zone called transitional zone, chondrocytes were more evenly distributed. The third zone, a radial or deep zone, contained chondrocytes that were organized in columns.

On the Safranin-O stain, the transitional zone was intensely stained, suggesting a high concentration of proteoglycans in this zone. The superficial zone was less stained, suggesting an inherent low concentration of proteoglycans. In the deep zone, high stainability of Safranin-O was noted, especially around the chondrocytes (pericellular region).

Histopathologic changes were seen on the femoral heads and acetabula. On the femoral heads and acetabula, roughening of the articular surface was noted (Figure 3-2-
F). Hypocellularity and diminished stainability on the H & E stain were also evident. A subchondral bone appeared denser and thicker compared to normal specimens.

On the Safranin-O stain, loss of proteoglycans from the upper part of the transitional zone was suggested by diminished stainability of this region.

**Analyses of Chondroitin Sulfates**

Two distinct peaks of CS-4 and CS-6 were identified following a peak of the standard marker (CA) on electropherograms from all samples. The averaged absolute amounts (µg) and standard deviations of CS-4 and CS-6 from the all samples were 2.60 ± 1.21 and 2.61 ± 1.01, respectively. The averaged absolute amounts (µg) and standard deviations of CS-4 and CS-6 from normal femoral cartilage (N=9) were 2.76 ± 1.28 and 2.69 ± 1.10, whereas those from degenerative cartilage (N=3) were 2.11 ± 0.92 and 2.37 ± 0.64. The proportion of CS-6 to CS-4 of normal cartilage was 1.02 ± 0.21, whereas that of degenerative cartilage was 1.24 ± 0.36. The difference was not statistically significant. The results were summarized (Table 3-1).

**Computed Tomography**

Computed tomography was performed in 3 cats. Two cats had radiographically and grossly normal hip joints. One cat had a radiographic sign of mild hip DJD with mild gross lesions on the caudal aspects of both femoral heads and both acetabula. On the CT images, a reliable evaluation of the joint conformation was impossible because of the low resolution of the images.

**Proton-MR imaging**

Magnetic resonance images of a normal femoral head were shown in Figure 3-1. On both sequences, articular cartilage appeared as intermediate signal intensity compared to the low signal intensity of subchondral bone and high signal intensity of joint fluid.
Joint fluid was hyperintense on both sequences but more pronounced on the T2-weighted FS FSE images. On both sequences, femoral cartilage was obscured by high signal intensity of joint fluid, especially at the caudal aspects. A subchondral bone appeared as a band of low signal intensity underlying articular cartilage and was much more clearly seen on the proton-density FS FSE images. On the proton-density FS FSE images, a thickened subchondral bone was also noted under the region where the round ligament attached to the femoral head, which was thought be a normal structure.

Magnetic resonance images of a femoral head with a radiographic sign of mild DJD were shown in Figure 3-2. On the ventrodorsal radiograph, a small osteophyte was seen on the dorsocranial acetabular rim. On CT images, the femoral head and acetabulum were blurred because of low resolution of the images. On the proton-density FS FSE image, a thickened band with low signal intensity was observed. This band corresponded to the thickened subchondral bone noted on the thin-section specimen. The thickened subchondral bone was also noted on the high-detail radiograph. Femoral cartilage was partially visualized on the cranial and caudal aspects, but obscured at the medial aspect and the caudal periphery of the femoral head by joint fluid. On the T2-weighted FS FSE images, contrast between cartilage and a subchondral bone decreased, making it hard to visualize the thickened subchondral bone. Similarly to the proton-density images, femoral cartilage was partially visualized on the cranial and the caudal aspects.

The SNRs of articular cartilage, bone marrow, and joint fluid were compared between the two sequences. The mean ± SD SNR of articular cartilage on the proton-density FS FSE images was 13.07 ± 6.52, ranging from 2.07 to 26.52, whereas that on the T2-weighted FS FSE images was 9.73 ± 5.78, ranging from 2.88 to 22.04. The
difference was not statistically significant. The mean ± SD SNR of bone marrow on the proton-density FS FSE images was significantly higher (9.34 ± 5.05, ranging from 4.38 to 19.02) than that on the T2-weighted FS FSE images (3.91 ± 1.96, ranging from 2.14 to 8.58). The mean ± SD SNR of joint fluid on the proton-density FS FSE images was 23.01 ± 8.90, ranging from 14.02 to 38.94, whereas that on the T2-weighted FS FSE images was 39.75 ± 25.76, ranging from 14.07 to 93.39. The difference was not statistically significant. The results were summarized in Table 3-2.

Sodium MR imaging

On the sodium MR images of the cat, only a distorted standard marker was observed. On the phantom images, three phantoms (1000mM, 500mM, and 250mM/L of sodium) were visualized. However, the phantom with the lowest concentration of sodium (125mM/L) was not visible. The sizes of the phantoms decreased as the concentration of the sodium decreased. The sodium MR images of the phantoms and the cat were shown in Figure 3-3. There was an excellent correlation between the signal intensities of the phantoms and the sodium concentrations ($r^2 = 0.9906$, $p<0.05$) (Figure 3-4).

Discussion

In the present study, MR images of hip joints were obtained in 12 cats in a high magnetic field (4.7-T). Normal articular cartilage was observed as a single intermediate-signal-intensity layer on the T2-weighted and proton-density FS FSE images. On the proton-density FS FSE images, contrast between articular cartilage and a subchondral bone and between articular cartilage and joint fluid was sufficient to visualize the anatomical structures in the hip joints of cats. Cortical bones of the femoral head and subchondral bones were well depicted with the proton-density FS FSE sequence. Contrast between articular cartilage and joint fluid was better on the T2-weighted FS FSE
images, owing to the higher signal to ratio of joint fluid on the T2-weighted FS FSE images.

Various MRI appearances of normal articular cartilage have been reported; however, laminated appearances have become a consistent observation. Thickness of articular cartilage with respect to image resolution explains why a laminated structure was not observed in hip cartilage in cats. A trilaminar appearance of articular cartilage was reported in human patellae on T1-weighted and T2-weighted SE images on 1.5-T (Modl et al., 1991). In that study, the imaging matrix and the field of view were 256 x 265 and 120 mm, resulting in the in-plane resolution of 470 µm². A trilaminar appearance of normal femoral condylar cartilage in human was also observed on FS 3-D SPGR images with a 1.5-T MRI unit (Disler et al., 1996). The imaging matrix and the field of view were 256 x 160 and 140 mm, resulting in the in-plane resolution of 550 x 880 µm². In these two studies, the thickness of patellar cartilage was not measured; however, in another study, the thickness of human femoral condylar cartilage was reported to be 4.65 to 5.01 mm (Jonsson et al., 1992). In contrast, in the present study, the imaging matrix and the field of view were 384 x 384 and 90 mm, resulting in the in-plane resolution of 230 µm², which was higher resolution (smaller pixel dimension) than the previous two studies. Although the measurement of cartilage thickness was not performed in this study, it was thought to be less than 0.2 mm based on the gross appearance. Therefore, even with high image resolution, a laminated appearance will not theoretically be observed in hip cartilage in cats.

Subchondral bones were clearly seen as a low to signal-void band underlying articular cartilage, especially on the proton-density FS FSE images. In the hip joints with
radiographic and gross pathologic signs of DJD, a thickened subchondral bone was apparent on both sequences, but more pronounced on the proton-density FS FSE images. This finding was considered to be significant, since thickening of subchondral bones and trabeculae were observed on MR images prior to radiographic signs of subchondral sclerosis and reported to be one of the changes seen in DJD (Stoller and Genant 1990).

Another reported abnormality seen in subchondral bones with DJD was increased signal intensity. In a study by Bredella et al. (1999) increased signal intensity was observed in a subchondral bone in a human patella with shallow ulceration or blisterlike swelling of cartilage. Although subchondral bone edema was thought to account for the change of signal intensity, the exact cause of this finding was not known. A change of signal intensity was not noted on the images in the cat hip joints in the present study.

Sodium MRI has been validated in \textit{in vitro} studies using bovine patellae (Shapiro et al., 2000, 2002). The technique has been also successfully applied to patellar cartilage (Shapiro et al., 2002), carpal cartilage (Borthakur et al., 2002), and intervertebral disks (Insko et al., 2002) in people. Shapiro et al. (2002) demonstrated that the sodium concentration in bovine cartilage could be reliably measured by sodium MRI. Furthermore, the FCD of cartilage calculated by the sodium concentration strongly correlated with the proteoglycan content that was measured by the standard dimethylmethylene blue assay. Therefore, sodium MRI was considered to be a useful technique in detecting early biochemical changes of cartilage.

Our initial intent was to correlate a change of a sulfation pattern of chondroitin sulfates to a change of a sodium concentration of degenerative cartilage. This objective was not achieved since sodium MR images were not obtained from the cat. Sulfation
patterns of proteoglycans have been known to associate with aging changes and degenerative changes of hyaline cartilage. In a study by Brown et al. (1998), the proportion of CS-6 to CS-4 decreased in horses with DJD. The increased amount of CS-4 was believed to reflect a reparative process of chondrocytes in degenerative cartilage. However, although the difference was not statistically significant we found that the proportion of CS-6 to CS-4 was higher in the feline articular cartilage with gross lesions than that in normal cartilage. This observation in the present study suggested that chondrocytes were not in a reparative process, which was also evident on the histological findings of hypocellular cartilage. Since all reported studies of sodium MRI were based on a correlation between sodium concentrations of cartilage and proteoglycan contents, a correlation study of sodium concentrations and sulfation patterns should add further information about a reparative status of articular cartilage.

Several reasons for the failure to obtain sodium MR images of hip joints can be considered. The most possible explanation would be the insufficient volume of sodium in the hip cartilage of the cat. The sodium concentration of articular cartilage was reported in a few studies. The sodium concentration of bovine patellar cartilage was measured by nuclear magnetic resonance spectroscopy (NMRS) and inductively coupled plasma emission spectroscopy (ICP) (Shapiro et al., 2000). The mean ± SD sodium concentrations obtained by NMRS and ICP were 335.62 ± 12.72 and 335.80 ± 16.21 mM/L, respectively. In the in vitro study by Shapiro et al. (Shapiro et al., 2002), a sodium concentration of enzymatically degraded bovine patellar cartilage was compared to that of normal cartilage. The sodium concentration of degraded cartilage was 261 mM/L, whereas that of normal cartilage was 316 mM/L. The sodium concentration of
normal cartilage in a human patella ranged from 140 to 350 mM/L, depending on the location and the depth; the concentration was high in the interior layers of cartilage and low at the edge of cartilage. In human carpal cartilage, Borthakur et al. (2002) reported that the sodium concentration in cartilage measured by sodium MRI was approximately 200 mM/L, which was significantly higher than those of ligaments and joint fluid (115-140 mM/L). Although the sodium concentration of hip cartilage in cats has not been reported, we assumed the sodium concentration of articular cartilage is similar to those of other species. However, the total amount of sodium in hip cartilage of cats was thought to be obviously less than those of bovine and human patellae due to the difference in thickness and volume. Moreover, on the sodium phantom images, we were able to see the phantom with a sodium concentration of 250 mM/L but not with 125 mM/L. The sequence we used for phantom imaging was a 3-D gradient echo pulse sequence which was more sensitive to a small amount of sodium signal than the sequence used for cat imaging.

The used coil in the present study had a diameter of 11.2 cm. This was an ideal size to place a whole cat in the coil. Yet, the area of interest, hip cartilage, was located relatively deep from the internal surface of the coil. This was the reason why we needed a relatively large coil. However, since a signal-to-noise ratio is closely related to coil geometry, it was also possible that the coil did not have enough sensitivity to detect a small amount of sodium signals from hip cartilage. To improve sensitivity of the coil, a small coil could have been used; however, a small coil had also a small area that it can detect a signal from. The anatomical differences between a patellar or carpal cartilage and hip cartilage are noticeable. Patellar and carpal cartilage locate relatively close to the
body surface, so the ideal signal-to-noise ratio can be readily achieved. In contrast, hip cartilage locates relatively deep from the body surface and the signal to noise ratio decreases when a birdcage coil is used due to the increased distance. Another alternative method would be a use of a surface coil. The positions of the hind limbs could be modified to decrease the distance between the coil surface and hip cartilage. For example, a use of a surface coil with a flog-leg position might be helpful to avoid superimposition of surrounding tissues over hip joints.

There were some limitations in the present study. First, we have collected all cat cadavers from a local animal shelter, so information of the cats (age, clinical history, diet, environment) was limited. We were not sure about the effects of post-mortem changes on the appearance of the MR images. However, since the all imaging was performed within 3 hours after euthanasia, we believed that the effects were minimum. To characterize the appearances of degenerative hip cartilage cats with advanced stages of DJD were needed. This was difficult to accomplish since cats in the shelter were usually young and advanced hip DJD was uncommon.

In conclusion, the proton-density FS FSE sequence provided adequate quality of the MR images of feline hip joints on the 4.7-T MRI unit. This sequence was particularly useful to depict subchondral bones, but articular cartilage was not fully visualized. The T2-weighted FS FSE images showed a better contrast between cartilage and joint fluid than the proton-density FS FSE images. Sodium MRI was not successful in feline hip cartilage most likely due to the insufficient volume of sodium contents in articular cartilage with respect to the low sensitivity of the used coil.
Table 3-1 Absolute amounts and proportions of chondroitin sulfate-4 and chondroitin sulfate-6 (mean ± SD).

<table>
<thead>
<tr>
<th>Gross grade</th>
<th>CS-4 (µg)</th>
<th>CS-6 (µg)</th>
<th>CS-6/CS-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>1.22 ± 0.1</td>
<td>1.73 ± 0.09</td>
<td>1.42 ± 0.06</td>
</tr>
<tr>
<td>Cat 2</td>
<td>3.82 ± 0.13</td>
<td>3.93 ± 0.16</td>
<td>1.03 ± 0.08</td>
</tr>
<tr>
<td>Cat 3</td>
<td>1.11 ± 0.1</td>
<td>1.90 ± 0.1</td>
<td>1.71 ± 0.09</td>
</tr>
<tr>
<td>Cat 4</td>
<td>1.62 ± 0.05</td>
<td>1.81 ± 0.2</td>
<td>1.12 ± 0.14</td>
</tr>
<tr>
<td>Cat 5</td>
<td>3.21 ± 0.15</td>
<td>3.21 ± 0.09</td>
<td>1.00 ± 0.07</td>
</tr>
<tr>
<td>Cat 6</td>
<td>5.32 ± 0.07</td>
<td>4.84 ± 0.15</td>
<td>0.91 ± 0.02</td>
</tr>
<tr>
<td>Cat 7</td>
<td>2.30 ± 0.07</td>
<td>2.84 ± 0.14</td>
<td>1.23 ± 0.03</td>
</tr>
<tr>
<td>Cat 8</td>
<td>2.78 ± 0.07</td>
<td>2.42 ± 0.08</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td>Cat 9</td>
<td>1.36 ± 0.01</td>
<td>1.29 ± 0.09</td>
<td>0.95 ± 0.06</td>
</tr>
<tr>
<td>Cat 10</td>
<td>3.42 ± 0.21</td>
<td>3.20 ± 0.11</td>
<td>0.94 ± 0.08</td>
</tr>
<tr>
<td>Cat 11</td>
<td>2.00 ± 0.02</td>
<td>2.01 ± 0.17</td>
<td>1.01 ± 0.1</td>
</tr>
<tr>
<td>Cat 12</td>
<td>2.98 ± 0.04</td>
<td>2.12 ± 0.11</td>
<td>0.71 ± 0.05</td>
</tr>
</tbody>
</table>

CS-4=chondroitin sulfate-4, CS-6=chondroitin sulfate-6.

Table 3-2 Comparison of the signal to noise ratios (mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Proton-density FS FSE</th>
<th>T2-weighted FS FSE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular cartilage</td>
<td>13.07 ± 6.52</td>
<td>9.73 ± 5.78</td>
<td>0.1986</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>9.34 ± 5.05</td>
<td>3.91 ± 1.96</td>
<td>0.0036</td>
</tr>
<tr>
<td>Joint fluid</td>
<td>23.01 ± 8.90</td>
<td>39.75 ± 25.76</td>
<td>0.0531</td>
</tr>
</tbody>
</table>
Figure 3-1 Photographs of a normal left femoral head.

(A) A proton-density FS FSE image of the femoral head. Articular cartilage is represented by a layer of intermediate signal intensity (arrow). Subchondral bone appears as a band of low signal intensity underlying cartilage (arrow heads). Hyperintense joint fluid obscures the caudal aspect of femoral cartilage.

(B) A T-2 weighted FS FSE image of the femoral head. Intermediate signal intensity of articular cartilage (arrow) and high signal intensity of joint fluid are seen. Subchondral bone is not well visualized. Tissue contrast is poor compared to a proton-density image.

(C) A high-detail radiograph of the femoral head. A subchondral bone is seen as a thin plate of radiopaque structure at the periphery of the femoral head. Slightly dense trabecular bone was noted under the region of the round ligament attachment and the caudal aspect.

(D) A photomicrograph of the femoral cartilage. A smooth articular surface and a zonal appearance characteristic of hyaline cartilage are seen. (H & E, ×100)
Figure 3-2 Photographs of a femoral head with a radiographically mild sign of DJD.

(A) A ventrodorsal radiograph of the femoral head. A small osteophyte (arrow) is noted on the dorsocranial aspect of the acetabular rim.

(B) A CT image. The detail of the femoral head and acetabulum was not delineated.

(C) A proton-density FS FSE image. A thickened subchondral bone is apparent.

(D) A T2-weighted FS FSE image. Femoral cartilage and the subchondral bone are less apparent because of the decreased contrast against surrounding tissues.

(E) A high-detail radiograph. A thickened subchondral bone is visualized.

(F) A photomicrograph of the femoral cartilage. Surface roughening, diminished stainability, hypocellularity, and a thickened subchondral bone are seen. (H & E, ×100)
Figure 3-3 Photographs of the sodium MR images.

(A) Sodium MRI of a cat. Only a distorted standard marker is seen.

(B) Sodium phantom images. Only three phantoms (left, 1000mM/L; middle, 500mM/L; right, 250mM/L) are visible. The size of the phantoms decreases as the sodium concentration decreases. The phantom with the 125mM NaCl solution is not visible.

![Sodium phantom calibration curve.](image)

The calibration curve was made by plotting the signal intensities obtained from three sodium phantoms and noise. Each data point represents 8 pixels from the phantoms and 28 pixels from noise.
CHAPTER 4
THE EFFECTS OF CARPROFEN ON FELINE ARTICULAR CHONDROCYTES CULTURED IN ALGINATE MICROSPHERES

Introduction

Degenerative joints disease (DJD) is the most common joint disorder in people (van Saase et al., 1989). Similarly, DJD is commonly seen in dogs (Olsson 1971). The disease has been characterized by progressive and irreversible degradation of articular cartilage and subsequent changes such as synovitis and bone remodeling (Johnston 1997). In a recent study, Hardie et al. (2002) reported that DJD was also commonly seen in geriatric cats. Furthermore, in another radiographic survey based on ventrodorsal radiographs of abdomen in 88 cats, the incidence of hip DJD was as high as 68.2% (60/88) (Kamishina and Miyabayashi 2002).

Non-steroidal anti-inflammatory drugs (NSAIDs) have been routinely used for a treatment of DJD in dogs (Johnston and Fox 1997). Carprofen, a propionic acid derivative, is one of the newest FDA approved NSAIDs for the treatment of DJD in dogs. Inflammatory mediators such as prostaglandins are produced by the cyclooxygenase 2 (COX-2), when tissue is injured or becomes inflamed. Carprofen inhibits a conversion of arachidonic acid into prostanoids (thromboxanes, prostaglandins (PG), and prostacyclin) by reversibly blocking the enzyme cyclooxygenase (COX); therefore, has anti-inflammatory, analgesic, and antipyretic effects (Fox and Johnston 1997).

The most significant side effects associated with NSAIDs occur due to the inhibition of the COX-1. The COX-1 pathway has a role in homeostatic function and
production of prostaglandins in gastrointestinal tracts, renal system, endothelial cells, and
platelets (Johnston and Fox 1997). Carprofen is similar to corticosteroids in that the two
drugs work on the same pathway and ultimately inhibit the synthesis of prostaglandins,
but to varying degrees. Corticosteroids differ from carprofen in that they inhibit
phospholipase A2, the precursor of arachidonic acid. Arachidonic acid is needed to
produce COX, the precursor of PGs (Johnston and Fox 1997). Since the inhibition of the
COX-1 induces various side effects, NSAIDs that have COX-2 selectivity are more
therapeutic and less toxic.

The COX selectivity of NSAIDs has been demonstrated. In dogs, Richetts et al.
(1998) reported that carprofen had the greatest potency of COX-2 inhibition among the
investigated NSAIDs. In the study, platelets were obtained from healthy beagles and
mixed with various NSAIDs. After incubation, thromboxane B2 was quantified by
enzyme immunoassay to estimate COX-1 production and inhibition by the NSAIDs.
Histocytoma cells were cultured and *Escherichia coli* endotoxin was added to the culture
media to induce PGE2 production. The NSAIDs were then added and enzyme
immunoassay was performed to quantify inhibitory effects of the NSAIDs on PGE2
production. The results revealed that carprofen had the highest selectivity against COX-2
(129-fold greater than against COX-1), whereas some other NSAIDs showed lower
selectivity against COX-2 (nimeslide: 38-fold; tolfenamic acid and meclofenamic acid:
15-fold). Other NSAIDs (meloxicam, flunixin, etodolac, and ketoprofen) did not show
COX-2 selectivity. This characteristic of carprofen was thought to contribute its low
incidence of main side effects such as gastropathy and renal toxicoses (McKellar et al.,
1990).
In contrast, Kay-Mugford et al. (2000) reported that the COX-2 selectivity of carprofen was lower than other tested NSAIDs (ketoprofen, meloxicam, and tolfenamic acid). In this study, a canine monocyte/macrophage cell line was used. Potency of the tested drugs was determined by calculating the concentration that resulted in 50% inhibition of COX activity (IC50). Selectivity was determined by calculating the ratio of IC50 for COX-1 to IC50 for COX-2. Meloxicam had the highest COX-2 selectivity (IC50 for COX-1 of 23.69 and IC50 for COX-2 of 1.93mg/ml), while carprofen had the least COX-2 selectivity (IC50 for COX-1 of 4.48 and IC50 for COX-2 of 2.56mg/ml).

More recently, Brideau et al. (2001) reported the COX-2 selectivity of carprofen, using whole blood samples obtained from horses, dogs, and cats. The COX-1 activity was determined by measuring the thromboxane B2 concentration in the samples by use of enzyme immunoassay. The COX-2 activity was determined by measuring the concentration of PGE$_2$ by use of radioimmunoassay. Potency and selectivity were calculated in a same manner as the study by Kay-Mugford et al. Carprofen was the weakest COX-2 inhibitor in horses, and showed little potency and selectivity for COX-2 in dogs and cats.

The effects of NSAIDs such as phenylbutazone, indomethacin, fenoprofen, and acetylsalicylic acid on proteoglycan synthesis in articular cartilage have been studied, using cultured chondrocytes or cartilage explants (Palmoski and Brandt 1980, 1983; Herman et al., 1986; Bassleer et al., 1992). The in vitro effects of carprofen on chondrocyte metabolism were studied more recently. Benton et al. (1997) reported the effects of carprofen on canine articular cartilage. In the study, at concentrations of 1 and 10mg/ml, there was a statistically significant increase in total glycosaminoglycan (GAG)
synthesis, while at concentrations of 20 and 50mg/ml the GAG synthesis significantly decreased. In the study, however, a chondrocyte monolayer culture system was used. In the monolayer culture system, it has been known that chondrocytes dedifferentiate to an atypical fibroblastic appearance and produce type I collagen rather than type II collagen (Aulthouse et al., 1989).

Three-dimensional chondrocyte culture systems are preferable, since newly synthesized proteoglycans are retained around the chondrocytes as seen in in vivo (Guo et al., 1989; Liu et al., 1998). There is only one study describing the effects of carprofen on chondrocytes, which were cultured in a three-dimensional culture system (Dvorak et al., 2002). In the study, canine chondrocytes were harvested from healthy humeral heads of 5 dogs and cultured in monolayer culture to amplify cell numbers. The chondrocytes were then suspended in 2 % agarose culture medium and cultured for 20 days under 6 different culture conditions; agarose only, agarose plus human recombinant interleukin (IL)-1β (20ng/ml), agarose plus carprofen (4µg/ml), agarose plus dexamethasone (1mg/ml), agarose plus IL-1β (20ng/ml) plus carprofen (4µg/ml), and agarose plus IL-1β (20ng/ml) plus dexamethasone (1mg/ml). The GAG contents in agarose gel, the GAG contents and PGE$_2$ contents in the liquid media, and matrix metalloprotease (MMP) -3 and MMP-13 contents in the liquid media were quantified on day 3, 6, 12, 20. The results showed that carprofen did not have positive effects on GAG synthesis by chondrocytes at any culture periods. Prostaglandins E$_2$ productions were significantly inhibited by carprofen and dexamethasone at all culture periods. In addition, carprofen and dexamethasone did not show significant protective effects against IL-1β in the MMP-
3 and MMP-13 productions. Therefore, carprofen seemed to have inhibitory effects on PGE$_2$ production, but not positive effects on GAG synthesis.

The articular cartilage matrix is comprised of an amorphous ground substance of proteoglycans within a meshwork of collagen fibers (Buckwalter and Mankin 1997). In proteoglycans, the predominant GAGs are chondroitin sulfates, mainly chondroitin sulfate-4 (CS-4) and chondroitin sulfate-6 (CS-6). The ratio of CS-6 and CS-4 changes with aging as well as DJD. The neonate articular cartilage showed the ratio of approximately 1:1, while cartilage from the aged person contained higher ratio of CS-6 and CS-4 than the neonates (Buckwalter et al., 1994). A similar age-related change in the ratio of CS-6 and CS-4 was observed in horses (Brown et al., 1998). In people with DJD, the ratio of CS-6 and CS-4 was lower in patients with DJD than ones without DJD (Mankin et al., 1971). This increased synthesis of CS-4 was thought to be associated with chondrocyte proliferation in a repair process of cartilaginous tissue. These reports suggested that changes in the amounts of CS-6 and CS-4 in the newly synthesized matrix could serve as an indicator of metabolic activities on chondrocytes.

The three-dimensional culture system has not been used in feline chondrocytes and the effects of carprofen on feline chondrocyte metabolism have not been studied. Therefore, the purposes of the present study were 1) to establish a three-dimensional feline articular chondrocyte culture system, and 2) to evaluate the effects of carprofen on feline chondrocytes, especially on the ratio of CS-6 and CS-4 of newly synthesized GAGs.
The hypotheses were: (1) feline articular chondrocytes would proliferate in alginate microspheres and synthesize chondroitin sulfates and (2) carprofen would have positive effects on feline chondrocyte proliferation and chondroitin sulfate synthesis.

**Material and Methods**

**Cat Cadavers**

Five domestic adult cat cadavers were collected from a local animal shelter immediately after euthanasia. There were 3 females and 2 males with a mean ± SD body weight of 3.2 ± 0.9 kg. All cats had radiographically normal hip joints. After the radiographic examinations, articular cartilage on the femoral heads and acetabula were grossly examined to confirm that the joints have no signs of degenerative changes.

The use of these cadavers was approved by the Institutional Animal Care & Use Committee at the University of Florida.

**Isolation of Articular Chondrocytes**

Cartilage tissues were aseptically sectioned from both femoral heads using a #22 surgical blade (Feather Industries Ltd., Tokyo, Japan) and placed in a Hank’s balanced salt solution (HBSS, Sigma Chemical Co. Ltd., St.Luis, MO). Sectioned cartilage was then diced under a laminar flow cabinet and digested in Ham’s F-12 medium (Sigma Chemical Co. Ltd., St.Luis, MO) containing 5% fetal bovine serum (FBS, Sigma Chemical Co. Ltd., St.Luis, MO), 0.4% pronase (Sigma Chemical Co. Ltd., St.Luis, MO), and 0.004% deoxyribonuclease (DNase, Sigma Chemical Co. Ltd., St.Luis, MO) for 90 minutes at 37°C in humidified atmosphere of 5% CO2 / 95% air. The digested tissues were centrifuged for 5 minutes at 1,500 rpm and washed three times with Dulbecco’s phosphate buffered saline (DPBS, Sigma Chemical Co. Ltd., St.Luis, MO). After washing the tissues they were further digested in Ham’s F-12 medium containing 5%
FBS, 0.012% collagenase (Sigma Chemical Co. Ltd., St.Luis, MO), and 0.004% DNase for 18 hours at 37°C in humidified atmosphere of 5% CO2 / 95% air. The digested cells were washed three times with HBSS. The freed cells were strained through a 70 µm filter (Cell Strainer, Becton Dickinson Labware, Franklin Lakes, NJ) to eliminate debris and kept in HBSS. Subsequently, the isolated chondrocytes were counted using a hematocytometer (Hausser Scientific, Horsham, PA) and the viability of the cells was measured by the Trypan blue exclusion technique. The chondrocytes were then suspended in Ham’s F-12 medium containing 10% DMSO and stored in liquid nitrogen until they were used for the monolayer culture.

**Monolayer Culture**

Frozen cells were thawed in a water bath at 37 °C for 1 minute and washed three times with HBSS. Thawed cells were counted and the viability was measured as described. Based on cell counting the chondrocytes were seeded in a culture flask at a concentration of approximately 600cells/cm² (9 x 10⁴cells/150cm²) and cultured in Ham’s F-12 medium containing 10% FBS, 2mM L-glutamin, and a 1% antimycotic/antibiotic solution (culture medium) at 37°C in humidified atmosphere of 5% CO2 / 95% air. The culture was continued until approximately 80% of the surface of the culture bottle was covered by the proliferated chondrocytes. The culture medium was changed every third day.

**Three-Dimensional Culture and Group Design**

The proliferated cells in monolayer culture were freed by trypsinization, using a 10% trypsin solution containing 0.02% EDTA (Sigma Chemical Co. Ltd., St.Luis, MO). The freed cells were washed three times with HBSS and the number and viability of the cells were measured as described. The cells were resuspended in DPBS containing 1.2%
of alginate (Kelton LV®, Kelco Company, Chicago, IL) at a cell concentration of 5 x 10^5/ml (Guo et al. 1989). The chondrocyte suspension was dropped into a 102mM CaCl_2 (Sigma Chemical Co. Ltd., St.Louis, MO) solution, 1 to 2 cm away from the surface of the solution through a 22-gauge needle. The microspheres were hardened in the 102mM CaCl_2 solution for 10 minutes. The microspheres were washed three times with HBSS and one time with culture medium. The microspheres were cultured in culture medium at 37°C in humidified atmosphere of 5% CO2 / 95% air for 24 days. Culture medium was changed every other day.

Four groups were made depending on the treatment conditions: group 1 (control) - 4µl of 100% benzyl alcohol/ml culture medium; group 2 - 1µg of carprofen/ml of culture medium; group 3 - 10µg of carprofen/ml of culture medium; and group 4 - 20µg of carprofen /ml of culture medium. Each group had approximately 285 microspheres in 15ml of culture medium.

Quantification of DNA Contents

Twenty microspheres were collected from each group on day 0, 12, 18, and 24. The microspheres were depolymerized in 1 ml of a 55mM sodium citrate (Sigma Chemical Co. Ltd., St.Louis, MO) solution containing 0.15M NaCl (Sigma Chemical Co. Ltd., St.Luis, MO) for 10 minutes at 37°C. The samples were then centrifuged at 14,000 rpm for 10 minutes. Collected cell pellets were stored at –20°C until the following analysis.

The samples were thawed at room temperature and digested with 200 ml of a 50mM Na2HPO4 (Sigma Chemical Co. Ltd., St.Luis, MO) solution containing 2.0mM L-cystein (Sigma Chemical Co. Ltd., St.Luis, MO) and 125mg/ml of papain (Sigma Chemical Co. Ltd., St.Luis, MO) for 8 hours at 60°C. Followed by papain digestion, 100 ml of the samples were mixed with 2ml of Hoechst dye solution (Sigma Chemical Co.
Ltd., St.Luis, MO) and fluorometric enhancement was measured by spectrofluorometry (TKO 120 Mini Fluorometer®, Hoefer Scientific, San Francisco, CA) at an emission range of 400 to 550 nm and an excitation wavelength of 365nm immediately after the reaction. A standard curve was made by measuring known concentrations (0, 30, 50, 100, 300, 500, 1000, 3000, and 5000 ng/ml) of highly polymerized calf thymus DNA (Sigma Chemical Co. Ltd., St.Luis, MO) (Kim et al., 1988). The DNA contents of the 20 microspheres were calculated based on the standard curve.

Analysis of Chondroitin Sulfates

One hundred microspheres were randomly selected from each group on day 18 and 24. The microspheres were washed with HBSS. After washing, the microspheres were placed in 10 ml of a 4M guanidine hydrochloride solution containing 50mM Tris-HCl, 10mM N-ethymalemid, 0.36 mM pepstatin A, and 1mM phenylmethylsulfonyl fluoride (PMSF) at 4°C for overnight (Oegema et al., 1979). The extracted solution was strained through a 70µm filter (Cell Strainer, Becton Dickinson Labware, Franklin Lakes, NJ). To eliminate other proteins, samples were subjected to the equilibrium density centrifugation. Cesium chloride (Fisher Scientific, Pittsburg, PA) was added to the samples at a concentration of 0.55 g/g of the sample solution. The samples were centrifuged for 48 hours at 40,000 rpm, 8°C, using a fixed angle rotor (Type T70.1, Beckman Instrument, Inc., Palo Alto, CA) in a centrifuge tube (Ultra-Clear Centrifuge tube 344322, Beckman Instrument, Inc., Palo Alto, CA). Newly synthesized proteoglycans were collected from the deepest layer in the tube (Maldonado and Oegema 1992). Desalination was performed with a PD-10 column. Then elution was performed with 0.02M Tris-HCl. Obtained samples were stored in -20°C until following digestion.
Extracted samples (275 µl) were digested with 5mU of chondroitinase ABC (Seikagaku America, Inc., Ijamsville, MD) containing 80 µl of 100mM Tris-HCl (Sigma Chemical Co. Ltd., St.Luis, MO), and 40 µl of cinamic acid (CA, Sigma Chemical Co. Ltd., St.Luis, MO) for 3 hours at 37°C. After 3 hours of digestion, the digestion was terminated by heating the sample in boiling water for 1 minute (Karamanos et al., 1995). Capillary electrophoresis (BioFocus®3000, Bio-Rad, Hercules, CA, U.S.A) was carried out to quantify the proportion of CS-4 and CS-6 in the digested samples. A fused silica capillary column (50µm i.d., 375µm o.d., 50cm length, Bio-Rad, Hercules, CA, U.S.A) was used. The digested samples were loaded under vacuum and electrophoresed for 15 minutes at 23°C, 15kV in a 40mM phosphate solution (Sigma Chemical Co. Ltd., St.Luis, MO), containing 40mM lauryl sulfate (Sigma Chemical Co. Ltd., St.Luis, MO) and 10mM sodium borate (Sigma Chemical Co. Ltd., St.Luis, MO) at pH 9.0. The eluant was monitored at 232nm (Carney and Osbone, 1991). Peak areas for both CS4 and CS6 were standardized by that of the standard marker (CA). Absolute amount of CS4 and CS6 were calculated from the standard curve which had been established by measuring known concentrations of serially diluted disaccharide samples (Seikagaku America, Inc., Ijamsville, MD) as described by Maeda et al. (Maeda et al., 2001).

**Statistical Analyses**

To compare the differences in cell proliferation among the four treatment groups, the DNA contents in 20 microspheres at day 12, 18, and 24 were divided by those of day 0. The divided values were compared among the four groups, using One-way ANOVA with p-value set at 0.05 (SAS Version 6.12). The absolute amounts of newly synthesized CS-4 and CS-6 on day 18 and day 24 were compared among the four groups, using One-way ANOVA with p-value set at 0.05 (SAS Version 6.12). If significant differences
were detected, Tukey’s studentized range test was performed to determine the significant difference among the tested groups (SAS Version 6.12).

Results

Cell Isolation and Viability

Articular chondrocytes were isolated from both femoral heads in 5 cat cadavers. The mean number of isolated chondrocytes from the femoral heads was $6.38 \times 10^5$, ranging from $6.0 \times 10^5$ to $6.85 \times 10^5$. The mean viability of isolated cells was 99.1%, ranging from 97.5% to 100%. After thawing the cells, the mean cell viability dropped to 78.8%, ranging from 60.6 to 100%. The chondrocytes were recovered from the monolayer culture after 10 days and the mean number of cells recovered was $6.8 \times 10^6$, ranging from $6.15 \times 10^6$ to $7.75 \times 10^6$. At this point, the mean cell viability was 91.9%, ranging from 78.0% to 98.3%. The results were summarized in Table 4-1.

Quantification of DNA Contents

In all groups, the DNA content decreased from day 0 to day 18. At day 24, the mean DNA content increased from day 18 in group 1 (control group) (528.2ng to 549.7ng) and group 2 (1µg/ml of carprofen) (541.3ng to 573.5ng), but continued to decrease in group 3 (10µg/ml of carprofen) (538.9ng to 529.4ng) and group 4 (20µg/ml of carprofen) (453.1ng to 411.4ng). At day 24, the DNA contents were slightly higher in group 2 than group 1 and slightly lower in group 3 than group 1. The DNA content of each group at day 12, 18, and 24 were divided by those of day 0, and the differences were tested. There were no significant differences among the groups. The results were summarized in Table 4-2.
Analysis of Chondroitin Sulfates

At day 18 and 24, absolute amounts of chondroitin sulfates were quantified by capillary electrophoresis. In all samples, only one peak was detected on electropherograms following a peak of the standard marker (CA). The peak was identified as CS-6 from the time the peak appeared and by adding 1µg of CS-4 and CS-6 (Seikagaku America, Inc., Ijamsville, MD) to the samples. Therefore, the mean amounts of CS-6 among the four groups were compared at day 18 and 24.

In all groups, the mean amounts of CS-6 decreased from day 18 to day 24. At day 18, the highest amount of CS-6 was noted in group 1 (1.62 ± 0.16µg) followed by group 3 (1.53 ± 0.08µg), group 4 (1.50 ± 0.05µg), and group 2 (1.47 ± 0.07µg). At day 24, group 1 (1.41 ± 0.08µg) and group 4 (1.41 ± 0.04µg) were the highest, followed by group 3 (1.37 ± 0.10µg) and group 2 (1.34 ± 0.15µg). A significant difference was not detected among the four groups. The results were summarized in Table4-3.

Discussion

The first purpose of the present study was to establish a three-dimensional feline articular chondrocyte culture system. As the results of the DNA measurements showed feline articular chondrocytes did not proliferate in alginate microspheres until day 18. The proliferation rate of chondrocytes in the alginate microspheres seemed to be affected by a use of monolayer culture. In our experience, feline chondrocytes proliferated much more rapidly when primary cells were encapsulated in microspheres. However, to carry out this experiment, a total of 5.6 x 10⁶ cells were needed in each cat. The cell number was multiplied to approximately 10-fold from the initial cell number (the mean cell number of 6.38 x 10⁵) with monolayer culture.
In a previous study by Maeda (Maeda 1999), canine articular chondrocytes proliferated in alginate microspheres from day 2 to day 20. The DNA contents increased from 112.4 ng (day 2) to 1,947 ng (day 20). In the study, monolayer culture was not performed and primary chondrocytes were suspended in the alginate solution at a concentration of 4 to 5 x 10^5/ml.

Gagne et al. (2000) reported that proliferation of chondrocytes in alginate microspheres was affected by the initial seeding density of the alginate microspheres. In their study, human articular chondrocytes were cultured in monolayer culture for three passages. Alginate microspheres were made with three different concentrations; high density (1x10^6/ml), intermediate density (1 x 10^5/ml), and low density (1 x 10^4/ml). The results showed that at 4 weeks of culture, the chondrocytes seeded at the low density had a nearly 3-fold higher median increase in cell number and a 6-fold greater level of sulfated GAG production.

In a study by Liu et al. (1998), human articular chondrocytes were reported to proliferate in alginate microspheres. Monolayer culture was performed for four passages and the chondrocytes were encapsulated in alginate microspheres at a concentration of 1 x 10^4/ml. Cell proliferation was confirmed by measuring [3H] thymidine incorporation. The chondrocytes proliferated for the first 25 days and then declined by the end of the culture period (day 70).

In the present study, the cell concentration of 5 x 10^5/ml was used since approximately 5 x 10^5 chondrocytes/100 microspheres were needed to successfully quantify CS with capillary electrophoresis (Maeda 1999). Although the data was based on canine chondrocytes, the DNA contents in 1 x 10^6 cells was reported to be 7ng
(Maldonado and Oegema 1992). At day 0, the mean DNA content from the 4 groups was 792.6ng with a range of 759.5 to 814.4ng. The amount of DNA was equivalent to $5.6 \times 10^5$ chondrocytes/ml, suggesting that the initial seeding density was reasonably close to the calculated value.

Lemare et al. (1998) reported that chondrocytes isolated from 1-2 months old rabbits restored their morphological and biochemical characteristics in alginate microspheres after 2 passages of monolayer culture. Reexpression of aggrecan and type II collagen genes was observed after 4 days of encapsulation. However, 2 weeks were necessary for total suppression of type I and III collagen synthesis, indicators of a monolayer phenotype. Nitric acid production by the encapsulated chondrocytes in response to IL-1β was also observed after 2 weeks of culture. In feline chondrocytes, although the chondrocytes morphologically restored in alginate microspheres, the cells might have not restored their biochemical characteristics. Therefore, the culture period may have to be extended in feline chondrocyte culture. Since restoration of gene expression characteristic of chondrocytes has not been studied in feline chondrocytes, it was warranted to ensure that the encapsulated chondrocytes fully regained their biochemical properties.

In the study by Maeda (1999), CS was not detected if the total DNA content was less than 748ng/20 microspheres. In the present study, the DNA content was higher than 748 ng/20 microspheres in all groups only at day 0. This suggested that the detected chondroitin sulfates were most likely those synthesized in the relatively early stages of the culture period. In addition, since chondrocytes produce CS-4 when they actively
proliferate as seen in physeal chondrocytes feline chondrocytes may have not synthesized enough CS-4 to be detected at day 18 and 24 when they had just started to proliferate.

The second purpose of the present study was to investigate the effects of carprofen on feline chondrocytes. Based on the results of the DNA measurements, carprofen did not show significant effects on cell proliferation. However, although the result was not statistically significant, the extent of the DNA decrease from day 18 to day 24 was greater in group 4 (20µg/ml) compared to other groups. In the study by Benton et al. (Benton et al., 1997), proliferation rates of canine chondrocytes decreased with concentrations of 20 and 50µg/ml of carprofen. In addition, cell viability was decreased in the group with 50µg/ml of carprofen. Although the differences were not statistically significant, the proliferation rates were also lower in the groups with the low doses of carprofen (1 and 10µg/ml) than a control group.

Effects of carprofen on feline chondrocytes in promoting synthesis of chondroitin sulfates were not observed at any concentration in the present study. In canine chondrocytes, although the major benefits of carprofen in DJD were believed to be its anti-inflammatory effects, direct positive effects on articular chondrocyte metabolism were observed at concentrations of 1 and 10µg/ml (Benton et al., 1997). Cartilage explants cultured in the presence of 1 and 10µg/ml of carprofen had significantly higher incorporation rates of $[^{35}S]$ sulfate into newly synthesized GAGs than a control group, while it was decreased in groups with 50 and 100µg/ml of carprofen. However, a different result was observed in the study by Dvorak et al. (Dvorak et al., 2002), where carprofen (4µg/ml) did not have significant effects on GAG contents in agarose medium at any time during the culture. The authors suggested that the effects of carprofen were
mainly attributed to its anti-inflammatory effects and positive effects on chondrocyte metabolism were limited.

In conclusion, a three-dimensional culture system is desirable to study chondrocyte proliferation and metabolism. However, further studies are necessary to validate the culture system for feline chondrocytes. First, it is important to confirm if the feline chondrocyte indeed restores its biochemical properties in alginate microspheres from a dedifferentiated monolayer phenotype. Second, there is a need to determine the ideal length of culture period, which allows chondrocytes to fully restore and maintain its properties in alginate microspheres. Third, the best seeding density for the feline chondrocyte should be determined, since cellularity of chondrocytes in vivo might be different among different species. Lastly, a trend of dose-dependent effects of carprofen on feline chondrocytes in promoting cell proliferation was observed. However, the positive effects of carprofen on chondroitin sulfate synthesis by feline chondrocytes seemed to be limited in the present study.
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<tr>
<th></th>
<th>Number of isolated cells</th>
<th>Viability after isolation</th>
<th>Number of thawed cells</th>
<th>Viability after thawing</th>
<th>Number of trypsinized cells</th>
<th>Viability after trypsinization</th>
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Table 4-2 DNA contents (ng) in 20 microspheres.

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<td>0</td>
<td>12</td>
<td>18</td>
<td>24</td>
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<tr>
<td>Group1</td>
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<td>807.2 ± 227.0</td>
<td>595 ± 232.7</td>
<td>538.9 ± 202.6</td>
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<td>759.5 ± 204.4</td>
<td>547.3 ± 240</td>
<td>453.1 ± 213.3</td>
<td>411.4 ± 190.8</td>
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</tbody>
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Table 4-3 Absolute amounts of CS-6 (μg/100 microspheres ± SD).

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<th>Day 18</th>
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<th>Day 24</th>
</tr>
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<td>Group 1</td>
<td>1.62 ± 0.16</td>
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<td>1.41 ± 0.08</td>
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<td>Group 2</td>
<td>1.47 ± 0.07</td>
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<td>1.34 ± 0.15</td>
</tr>
<tr>
<td>Group 3</td>
<td>1.53 ± 0.08</td>
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<td>1.50 ± 0.05</td>
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LIST OF REFERENCES


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BIOGRAPHICAL SKETCH

Hiroaki Kamishina was born on December 1, 1971, in Oita, Japan. He received his Bachelor of Veterinary Medical Sciences degree from Rakuno Gakuen University, Japan, in March 1996. He then worked in a small animal practice for four years. After that, he came to the University of Florida and did research on canine and feline chondrocyte cultures. From January 2002 to present, he has been a master’s student in veterinary medical sciences at the University of Florida. He also works as a research assistant under the guidance of Dr. Takayoshi Miyabayashi.