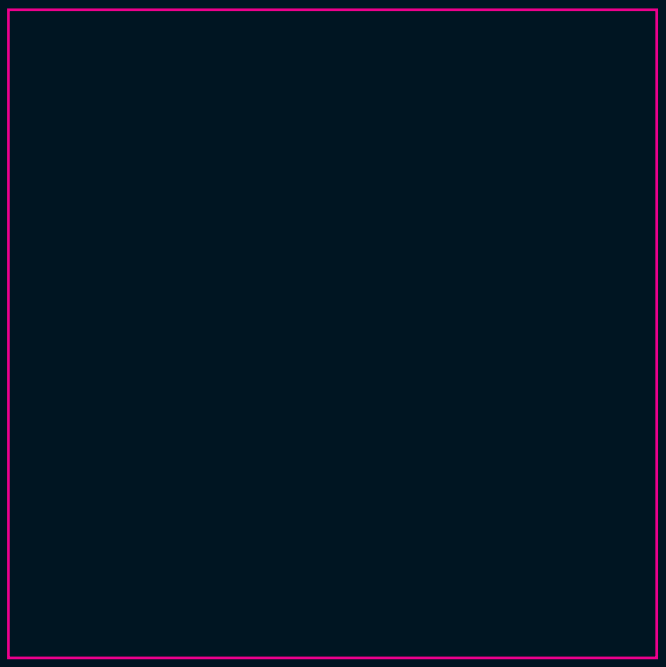
SYMPOSIUM PROCEEDINGS

Genova,
25th to 27th April
2005

Hill's European Symposium on Osteoarthritis and Joint Health



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Global Leader in Pet Nutrition

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Introduction

We are delighted to present the Proceedings of Hill's European Symposium on Osteoarthritis and Joint Health held in Genova April 25 to 27, 2005. This Symposium, the 9th in a series of consecutive Hill's European Symposia, offers an ideal opportunity to discuss the latest developments in the field of osteoarthritis in dogs with some of the world's leading authorities on the subject.

Osteoarthritis has been estimated to affect up to 20 per cent of dogs over one year of age. A relatively new concept in the treatment of osteoarthritis is the use of omega-3 fatty acids, especially eicosapentaenoic acid (EPA). Recent studies have shown that feeding Prescription Diet* Canine j/d* significantly improves the ability of dogs with osteoarthritis to rise from a resting position, to run, play, and walk.

I would like to thank all the speakers for helping to ensure the success of the Symposium and hope that you find these Proceedings an interesting and informative basis for future discussion of what is a significant area for the veterinary profession.

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Canine Osteoarthritis – Initiating Factors

INTRODUCTION

"Diseases do not exist; disease processes do".

Osteoarthritis (OA) is the most common arthropathy of man and animals and is especially prevalent in dogs. OA should be considered as a disease process; the final common pathway for joint failure. In dogs, it is usually possible to identify the primary initiating cause. That said, given the same initiating cause, there can be individual variation in the site and severity of OA that develops as explained in FIGURE 1.

Common causes of OA include joint instability (laxity or ligament failure), joint incongruity and intra-articular fracture. There are many potential initiating causes of OA in dogs and this paper will discuss the 'the big three' – hip dysplasia, elbow dysplasia and cranial cruciate ligament failure.

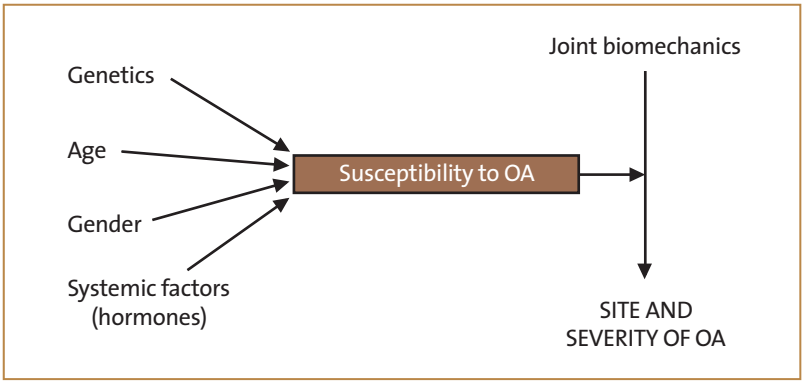

Figure 1. Model of Osteoarthritis *(adapted from British Journal of Rheumatology 1994; 33: 201-204 [1]).*

HIP DYSPLASIA

Hip dysplasia can be defined in terms of 'excessive laxity of the hip joint' but one must bear in mind that this is a dynamic process with temporal variability.

In recent years, much research work has been done to quantify hip laxity and to further refine the relationship between laxity of the coxofemoral joint and osteoarthritis. However, a clear distinction must be made between the use of measured laxity to estimate the relative risk of coxofemoral osteoarthritis in breed populations (e.g. PennHIP®) and the clinical relevance of laxity to the individual dog. This lecture will concentrate on the relevance of hip laxity to the individual dog.

Natural history of hip dysplasia

Pathology

Histopathological studies of puppies with a high prevalence of hip dysplasia have shown that the earliest pathological change evident is in the teres (round) ligament of the femoral head and the joint capsule. The function of these tissues is thought to be important in maintaining close articulation between the femoral head and acetabulum. Reaction of the subsynovial layer of the joint capsule in developing hip dysplasia include fibroblastic proliferation and increased extracellular matrix production (fibrosis).[2,3] The extracellular matrix of the subsynovium is mainly composed of collagen. Previous studies have highlighted a change in the composition of collagen in the joint capsule in dogs with hip dysplasia, namely an increase in the type III to type I ratio. However, it is likely that this alteration in composition is a secondary response since deposition of type III collagen is a normal repair response in fibrous tissues, perhaps because it forms rapid cross-links and can precariously provide strength to the matrix.[4,5] However, repeated injury to the ligament and joint capsule is likely to result in gradual weakening and stretching of the tissues (FIGURE 2). Interestingly, the ligamentum teres has similarities with the cranial cruciate ligament; both are intra-articular ligaments with a poor propensity to heal.[6] The resulting fibrosis of the joint capsule appears to gradually reduce passive hip laxity. However, the period of laxity may result in abnormal development of the acetabulum and femoral head leading to poor joint congruity (FIGURE 2). In addition, abnormal loading of articular cartilage may result in depletion of proteoglycan and disruption of the type II collagen network. These biochemical changes are the hallmark of osteoarthritis. Once this process has been initiated, it appears that it is progressive, although to a variable degree.

Clinical signs

The clinical signs of hip dysplasia generally become evident to pet owners at approximately five to nine months of age. Since passive laxity of the hip can be reliably measured at four months, this would suggest that laxity itself is not the primary cause of pain.[7] One might hypothesize that the consequences of hip laxity, namely, damage to the joint margins and synovitis, are the causes of pain at this stage of the disease. Recent data on the efficacy of denervation of the joint capsule in young dogs might support this theory.[8]

The results of conservative management of hip dysplasia in young dogs are not well documented. A rather old study suggested that approximately 75 per cent of dogs treated conservatively achieved a good functional outcome.[9] This suggests that in terms of clinical signs, the natural history of hip dysplasia is that the fibrosis and remodelling of the hip joint eventually result in reduced pain for the majority of animals.

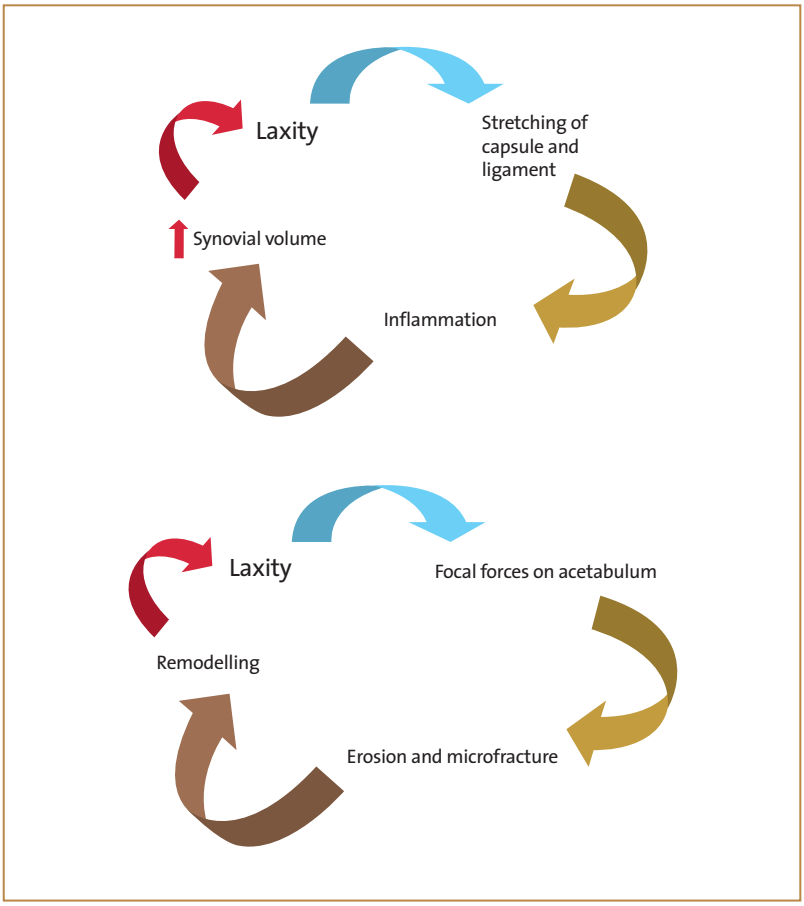

Figure 2. Disease processes in canine hip dysplasia.
This figure shows how joint laxity may promote disease progression.

Anecdotal evidence indicates that a number of animals experience recrudescence of pain later in life, presumably because of progressive arthritis. The pain mechanism at this stage is not well understood and could emanate from the synovium, subchondral bone, or both.

The pathological and clinical natural history of hip dysplasia must be borne in mind when considering individual animals. For example, ask the rhetorical question: what stage in the pathological process is this animal at?

Assessment of hip laxity

There are several methods to assess hip laxity. Some of these are designed for assessment of individual dogs and some are designed as measures to be used in breeding programmes.

Palpation tests - Ortolani

The Ortolani test is probably the most widely used and most studied of the canine hip palpation tests. The test can be performed with the anaesthetized, or heavily sedated, dog in lateral or dorsal recumbency, although the author prefers the latter. The test relies on manual force to subluxate the femoral head, with gradual abduction of the hip allowing eventual relocation of the femoral head; this relocation is accompanied by a 'clunk' as the femoral head slips over the dorsal acetabular rim. The sensation felt by the clinician during this relocation can provide some information on the integrity of the dorsal acetabular rim. A positive Ortolani sign is indicative of hip laxity but there is a threshold to this test and it is a categorical or ordinal discontinuous test (i.e. positive/negative or mild/moderate/severe) that provides limited information.

Studies have been performed on the relationships between the Ortolani test in individual dogs and radiographic measures of hip laxity.[10] There is at best, a moderate correlation between the Ortolani test and radiographic measures. In addition, in the absence of osteoarthritis there is a linear relationship between the Ortolani sign and passive hip laxity but this is not so when osteoarthritis is present.[10] This demonstrates the need to combine methods of laxity assessment.

Imaging methods

Radiographic techniques

The traditional radiograph of the hip joints (ventrodorsal, hip extended) gives little information on hip laxity. Extension of the hip causes tension in the joint capsule and resultant medial displacement of the femoral head in a lax hip; thus laxity is minimised using this view.[11]

Radiographic techniques to assess laxity in a more neutral hip position include the distraction index (DI) as used in the PennHIP® programme, the subluxation index (SI) and the dorsolateral subluxation (DLS) technique.[7,11-16] There is ongoing debate as to which of these methods is preferred but the body of information and validation is greatest for DI.

Recent work with DI has highlighted breed differences in the tolerance for passive hip laxity. For instance, as a breed, the Rottweiler was shown to be more tolerant of passive laxity than the German Shepherd Dog.[17] One must remember that these data are derived from studies of populations and the 95 per cent confidence intervals can be quite large. Thus it becomes difficult to make predictions for individual dogs. That said, in published studies, DI was the strongest predictor for development of osteoarthritis of the background and disease variables studied. Furthermore, the current data do suggest that on an individual patient basis, a DI of less than 0.3 indicates that a dog is very unlikely to develop osteoarthritis.

Ultrasonographic techniques

The use of ultrasound for assessment of hip laxity has been limited. Imaging at six to 16 weeks of age appears to be optimal.[18] Recent data indicate that ultrasonography suffers from problems with reliability.[19]

Arthroscopy

Arthroscopy of the canine hip was reported many years ago but has recently received repeat attention.[20] Arthroscopy is teaching us more about the lesions within painful, lax hips of young dogs. Lesions of the teres ligament, the joint capsule and the labrum can be inspected. In addition, arthroscopy, in combination with radiofrequency may provide us with opportunities to treat hip laxity; this has been reported in human patients and has been used widely in the shoulder joint.[21,22] However, caution is urged, since thermal shrinkage of joint capsule leads to immediate reduction in structural and material properties of the tissue and the capsule must be protected from physiological loads during repair.

Summary

Hip laxity is a common feature of hip dysplasia. At present the primary use of a measure of hip laxity is in breeding programmes. As data on the meaning of hip laxity in individual patients is accrued, it might be possible to develop protocols for clinical management of patients that incorporate assessment of hip laxity in to the treatment model.

ELBOW DYSPLASIA

Elbow dysplasia is a very common, probably underdiagnosed, group of developmental orthopaedic diseases of the canine elbow joint. Probably underdiagnosed because it can be difficult to confirm the diagnosis in young dogs using traditional modalities. Although the term elbow dysplasia has been adopted internationally, there is no firm scientific evidence that this is truly a dysplasia in the strictest sense. In addition, the grouping of three or four disease entities under one umbrella term, may not be appropriate with our current understanding (or lack of understanding) of the aetiopathogenesis of these diseases. Although the term elbow dysplasia includes four diseases (fragmentation of the medial coronoid process, osteochondritis dissecans [OCD] of the medial humeral condyle, ununited anconeal process and elbow incongruity) this discussion will focus on the most common of these, namely fragmentation of the medial coronoid process (FCP).

Aetiology

FCP is thought to be multifactorial although there is greatest evidence for a genetic basis for the disease. Several studies have addressed the inheritance for elbow dysplasia using radiographic scoring to define phenotype and, since FCP constitutes approximately 97 per cent of elbow dysplasia cases, it is probably fair to say that these studies are concerned with the inheritance of FCP.[23-26] These studies have come from Scandinavia, UK, Continental Europe, Australia and North America. Whilst the published heritability estimates for FCP differs somewhat between these papers, there is a general consensus that FCP has an inherited component that is significant enough to indicate that breeding strategies could be useful in controlling the disease. It seems most likely that the disease is a polygenic trait but where there is debate as to whether the mode of inheritance is maternal (mitochondrial DNA) or sex-linked (X-chromosomal).

With the rough map of the canine genome now almost complete, there is the opportunity to conduct whole genome screening in molecular genetic studies in an attempt to identify genes associated with FCP. The design of these studies will depend on the populations available for study. 'Linkage analyses' will be possible if DNA is available from extended pedigrees containing affected individuals and this is a powerful method for detecting the relevant genes. 'Disease association studies' are less powerful but more achievable since detailed pedigree information is not required. The UK DNA archive (http://pcwww.liv.ac.uk/DNA_Archive_for_Companion_Animals/) is currently collecting samples for this type of study.

Pathogenesis of fragmented coronoid process

Gross and histopathological studies of FCP have suggested that FCP is a fracture rather than an osteochondrosis. Why should the FCP fracture? It is possible that this is a stress fracture. It is also possible that there are excessive forces on the medial coronoid process caused by abnormal joint shape or loading. In the Bernese Mountain Dog, it has been suggested from post mortem studies that there may be incongruency 'step' between the radial head and the medial coronoid process.[27,28] In addition, it has also been suggested that there may be humero-ulnar incongruency such that the diameter of the humeral condyle is larger than that of the semilunar notch of the ulna, causing excess load at the margins of the ulnar notch.

Diagnosis of fragmented coronoid process

Diagnosis of FCP can be challenging. The signalment can be helpful in that the breeds commonly affected include Labrador and Golden Retrievers, Rottweiler, Bernese Mountain Dog, Mastiffs and Newfoundland. A typical age for presentation is five to nine months but some dogs are older (9-18 months) and, occasionally, much older (e.g. four to six years).

Clinical signs include mild-moderate thoracic limb lameness and inactivity stiffness. Affected dogs may also typically stand with the lower thoracic limbs externally rotated. Because the disease is usually bilateral, there may be symmetry to the clinical signs and lameness may not be

obvious. Bilaterally painful dogs may exhibit a stilted thoracic limb gait. There is pain on elbow joint manipulation, particularly full extension, external rotation, and full flexion combined with internal rotation. Some affected joints have a palpable effusion caudolaterally but this is the exception rather than the norm.

Radiography is the traditional method of evaluating elbow joints. Of course, this does not make it the best method. Mediolateral (flexed, neutral and extended) and craniocaudal views of the joint can provide information on secondary osteophytosis, or whether there is OCD of the medial humeral condyle, or ununited anconeal process; in the majority of joints, osteophytosis is the only change noted. One major problem with relying on osteophytosis as a marker of FCP is that it can take weeks or months for this secondary change to occur. Oblique radiographs have been investigated as an alternative method to detect fragmentation but since the position of the fragment is usually on the lateral aspect of the medial coronoid, adjacent to the radial head, it is unlikely that oblique views will add useful information. The degree of osteophytosis can be graded (as in the international elbow working group scheme) but the relationship between osteophyte grade and FCP is poorly documented. One study did suggest a correlation between osteophyte grade and degree of cartilage erosion.

Advanced imaging techniques can also be used to evaluate elbow joints but the limited availability of these is a problem. Computed tomography has been shown to be sensitive to detection of FCP but there is limited information on the use of MRI.

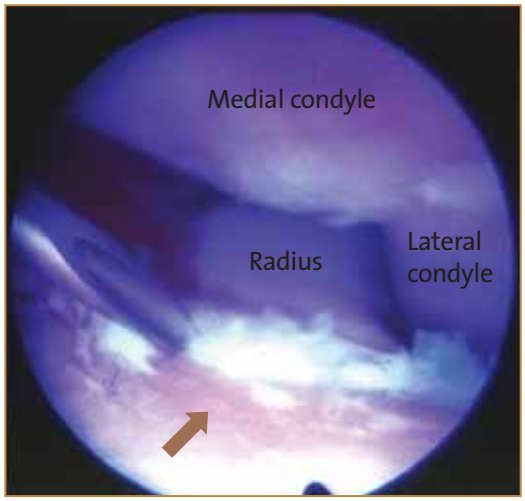

FIGURE 3. Arthroscopic view of the medial coronoid process (foreground) showing erosion of articular cartilage and exposure of subchondral bone (arrow).
A 20 gauge hypodermic needle is entering the field of view (left) to probe the surface of the medial coronoid process.

Arthroscopy is probably the gold standard for diagnosis and staging of FCP (FIGURE 3). A medial portal with a 1.9mm or 2.4mm arthroscope is the standard approach. Arthroscopy allows for the detection of non-displaced fragments as well as cartilage fissures and chondromalacia. Training and practice of this technique are important to develop an appropriate level of expertise. Arthroscopy also facilitates operative management of the condition.

CRANIAL CRUCIATE LIGAMENT FAILURE

The cranial cruciate ligament (CCL) is a critical stabiliser of the stifle joint. Degeneration and rupture of the CCL is the most common disease affecting this joint but the aetiology is uncertain. Certain common breeds of dog (e.g. Labradors) are predisposed to degeneration of the CCL leading to osteoarthritis (OA); others (e.g. Greyhounds) rarely suffer this injury. By comparing these two breeds we have investigated factors that may be relevant to understanding why the Labrador CCL is prone to failure, whereas the Greyhound CCL is not. [29-31]

In order to fulfil its biomechanical role in the stifle, the CCL needs to operate under tension. Therefore, its biochemical composition is adapted for this function. The CCL is composed of specialised fibroblasts 'ligamentocytes' and they maintain the extracellular matrix of collagen (mostly type I), elastin, proteoglycans and other glycoproteins. The composition of connective tissues is maintained by a balance between collagen and proteoglycan synthesis and their degradation by matrix metalloproteinases (MMPs) and ADAM-TS enzymes.

Damage to tissues, such as the CCL, results from or in an imbalance between these processes. Biological and mechanical factors can drive this imbalance.

In the 1980s, Vasseur and colleagues described histological changes in CCLs from dogs that suggest an age-associated degeneration of the tissue; an effect more marked in larger dogs. We have recently found that the CCL matrix of Labradors differs markedly from that of Greyhounds, both in terms of composition and in markers of collagen turnover, in the absence of gross joint pathology. [32] Furthermore, we have shown that grossly normal CCLs from

Labradors have increased glycosaminoglycan (GAG) content and increased MMP activity, together with an increased ratio of immature to mature collagen cross-links. These findings indicate an increased turnover in the CCL of the predisposed breed. Histologically, some Labrador CCLs show degeneration and chondroid metaplasia; chondroid metaplasia is characterised by a focal accumulation of chondrocyte-like cells, which express molecules normally associated with cartilage matrix. Cartilage matrix is designed to operate under compression, not tension and so clearly, a cartilage-type matrix is inappropriate and may render the CCL susceptible to rupture.

The differences in CCL matrix could be constitutive or induced. In order to investigate this issue, we have undertaken studies of the gait of the normal Labrador and Greyhound in order to estimate the forces acting through the stifle joint. If the CCL of the Labrador is under greater load during normal activity, this might explain why it tends to degenerate. We have used a combination of kinetic and kinematic gait analysis techniques, together with morphometric studies, to estimate the forces, moments and powers acting through the stifle joint in Labradors and Greyhounds.[29] These studies show striking differences between the two breeds. For example, it appears that in the Labrador, the gastrocnemius muscle acts to a greater degree to draw the femur caudally on the tibial plateau during stance phase. This could have the effect of increasing the load on the CCL.

Evidence is also accumulating to support the hypothesis that the endocrinological status of animals is important in maintaining connective tissue homeostasis. Neutered dogs are predisposed to CCL degeneration, as are human female athletes. In addition, oestrogen has been shown to affect CCL turnover in rabbits, but leptin and growth hormone (GH) are other candidates for hormonal control of connective tissues.

Leptin is produced from white adipose tissue. Obese dogs have higher levels of circulating leptin. Recent studies in humans have shown increased levels of leptin in synovial fluids of obese patients. Of course, leptin and GH might act through their link with body weight and composition, thereby affecting the mechanical load to which the CCL is exposed. However, there is sufficient evidence to support the proposal that hormones can act directly on connective tissue cells.

We have recently shown that leptin can modulate the activity of canine CCL cells *in vitro*, causing alteration of collagenase activity. In these recent experiments, CCL cells were isolated using bacterial collagenase digestion and maintained as monolayer cultures. Following exposure of cultures to human recombinant leptin, aliquots of medium from these cultures were assayed for MMP activities. Gelatin zymography was used to semi-quantitatively assess MMP-2 and -9 expression in cell conditioned medium and a fluorogenic substrate assay was used to measure latent and active levels of collagenase expression. Reverse transcriptase PCR was used to investigate the expression of the functionally active form of the leptin receptor isoform (OBRb) in CCL cDNA.

Canine CCL cells were shown to express MMP-2 in culture. However, as found from previous studies on whole tissue, there was no expression of MMP-9. MMP -2 and -9 levels in leptin treated cells showed no significant change from untreated controls. A significant change in collagenase activity in response to leptin treatment was observed in the cell cultures. These data suggest that there is a role for leptin in the metabolism of the CCL. To support these findings, the PCR amplification has yielded a product with molecular weight corresponding to OBRb, the identity of which will be confirmed by sequencing.

The connectivity of CCL cells may also be critical to their survival. It has recently been shown that canine tenocytes are connected by a pericelluar

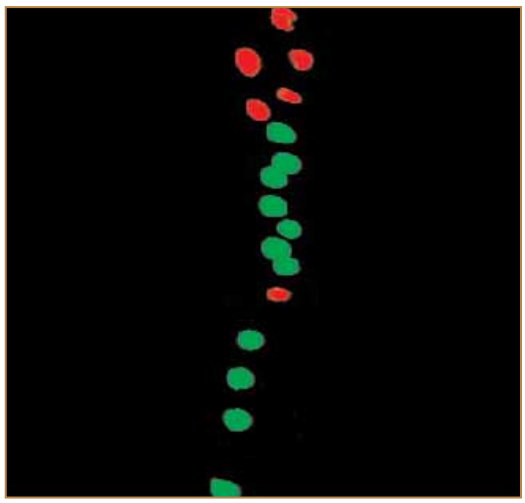

Figure 4. Linear canine CCL cell array showing viable (green) and non-viable cells (red).

array containing type VI collagen, versican and fibrillin-2.[33] We have shown that canine CCL cells are connected in a linear fashion by a collagenase-resistant matrix. Severance of this connectivity appears to result in cell death (FIGURE 4).

In summary, we believe that a greater understanding of CCL degeneration will provide a better basis for treatment and prevention. Our studies are ongoing and we aim to explore the link between mechanical load and biological response of CCL cells.

SUMMARY

By considering the 'big three' diseases of canine joints, we have discussed the initiating factors for development of OA in dogs. It is likely that over the next few years the underlying mechanisms will be further elucidated such that we may be able to target therapies at the primary cause of OA as well as the disease process itself.

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Eicosapentaenoate Supplementation Abrogates Canine Articular Cartilage Degradation in '*in Vitro*' Explant Culture Systems

INTRODUCTION

Canine models have been widely used to study the biological and molecular mechanisms involved in cartilage matrix degradation as the pathogenesis of degenerative joint diseases progress.[1-4 AND REFERENCES THEREIN] Previous research from our laboratory has shown that prior exposure of articular cartilage to omega-3 polyunsaturated fatty acids (n-3 PUFAs) could cause the inhibition of enzymes involved in cartilage matrix degeneration.[5,6] These studies use cartilage tissue explants exposed to a variety of different cytokines, growth factors and chemical mediators that activate degradative enzymes and metabolic factors that induce and propagate cartilage matrix degradation.[7,8 AND REFERENCES THEREIN] The objectives of this study were to determine which catabolic stimulants were the most suited for induction of cartilage matrix (proteoglycan) catabolism in a canine model explant culture system and then to investigate the potential for n-3 PUFAs to modulate these degenerative processes in canine cartilage metabolism.

MATERIALS & METHODS

Full-depth articular cartilage was obtained from the knee (stifle) joints of dogs that were euthanased for other medical reasons with ethical consent. Tissue from three groups of dogs was analysed. Pooled cartilage from knee joints of dogs of variable age with no overt joint pathology were used in studies involving the evaluation of *in vitro* explant culture systems that mimic cartilage degradation in degenerative joint disease. Cartilage from this same group of dogs was used in our initial studies investigating the effects of n-3 PUFAs on modulating cartilage catabolism in these culture systems. There was also a third group of five older dogs of the same breed (beagle dogs; three males and two females, aged 12-14 years) who had been fed the same diet for the past two years.

I. Evaluation of *in vitro* culture systems that mimic cartilage degradation *in vivo*

Canine articular cartilage from four dogs with no overt pathology was pre-cultured in Dulbecco's modified Eagle's medium (DMEM) containing ten percent foetal calf serum (FCS). Cultured explants were washed in serum-free DMEM and maintained for four days in 24-well tissue culture plates containing one millilitre of serum-free DMEM with or without added catabolic agents.[7,8] The catabolic agents studied were retinoic acid (RA) (10^{-6} M), recombinant human IL-1α (10-100 µg/ml), recombinant human IL-1β (10-100 µg/ml), recombinant TNF-a (100 ng/ml) and recombinant human oncostatin M (OSM; 50 ng/ml). After four days culture media was collected and analysed for lactate content, sulphated glycosaminoglycan (GAG) content and the presence of cartilage aggrecan catabolites generated by aggrecanase or matrix metalloproteinase (MMP), after Western blot analyses using monoclonal antibodies (mAb) BC-3 and BC-14, respectively (Abcam: www.abcam.com).[7]

II. Effects of n-3 PUFAs on cartilage catabolism

Cartilage was obtained individually from a second group of four dogs of unknown age with no overt joint pathology and was pre-cultured as described in section (I) above. Cultured explants were washed in serum-free DMEM and then maintained for five days in serum-free DMEM containing 100 or 300 µg/ml of one of the following n-3 PUFAs: a-linolenate (ALA; 18:3n-3; Sigma L2376) eicosapentaenoate (EPA; 20:5n-3; Sigma E2011) or docosahexanoate (DHA; 22:6n-3; Sigma D2534) as previously described.[5,6] – Note: individual PUFAs were pre-incubated for 16 hrs at 37°C and dissolved in a solution of 3.5 mg/ml fatty acid-free bovine albumen (Sigma, A8806) before being added to the cartilage explants at the concentrations described above. After five days exposure to the PUFAs this culture media was removed and the cartilage explants were washed. The culture media was then replaced by fresh serum-free medium (without PUFAs) supplemented or not with 50 ng/ml human recombinant OSM (Sigma, O9635) and cultured for a further four days and the media analysed as described above.

III. Effects of n-3 PUFAs on cartilage metabolism in old dogs of the same breed

Pooled cartilage was obtained from the right and left knees of four individual dogs (two males and two females, 12-14 years old). At arthrotomy of the fifth dog (male, 14 years old) the right knee was found to have overt signs of joint pathology in the articular cartilage; therefore, cartilage obtained from the individual right and left knee was cultured separately, as described below. Articular cartilage from all five dogs was pre-cultured before being exposed for five days to the n-3 PUFAs EPA and ALA (both at 100 & 300 µg/ml), and subsequently cultured for an additional four days (without n-3 PUFAs) either with or without exposure to 50 ng/ml OSM as described in (II). The media was then analysed as described in section (I) above.

RESULTS

Evaluation of *in vitro* culture systems that mimic cartilage degradation *in vivo*

Lactate analyses indicated that exposure to the catabolic agents and/or omega-3 PUFAs did not affect cell viability (results not shown). FIGURE 1 shows the GAG released into the explant culture media from cartilage slices that were cultured in the absence (control) or presence of catabolic stimulants (IL-1 α & β, TNF-α, OSM and RA).

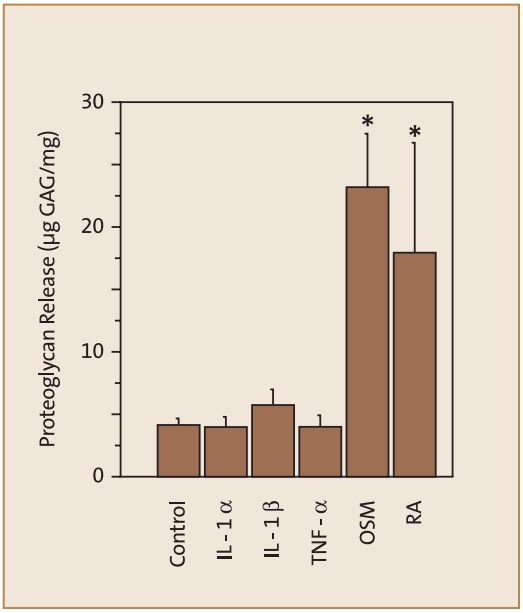

Figure 1. Proteoglycan [= sulphated glycosaminoglycan (GAG)] release (µg GAG/mg) *into the culture medium of canine articular explant cultures that were either untreated (control) or exposed to the catabolic stimulants IL-1 α, IL-1 β, TNF-α, Oncostatin M (OSM) or retinoic acid (RA).*

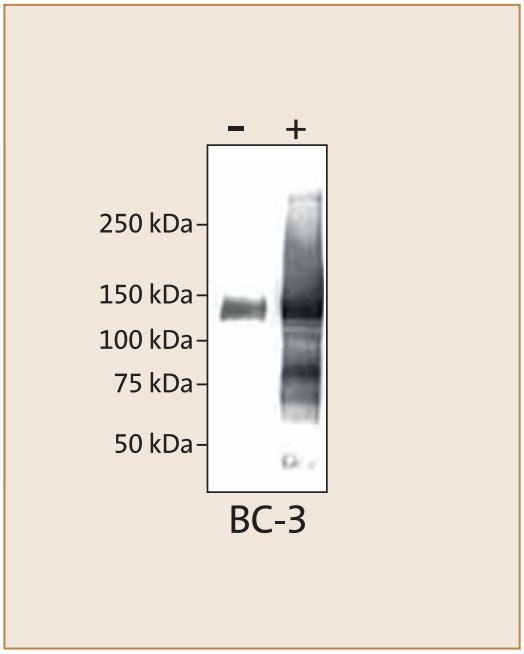

Figure 2. Western blot analyses using monoclonal antibody BC-3 *of media from control (-) and OSM (+) exposed cultures. BC-3 antibody recognises aggrecanase-generated aggrecan catabolites.*

Significant increases in GAG release were only observed when canine articular cartilage was exposed to leukaemia inhibitory factor (LIF), OSM and RA. These analyses indicated that OSM and RA exposure appeared to cause the greatest release of GAG into the culture media (FIGURE 1).

FIGURE 2 shows the results of a representative BC-3 Western blot, which detects aggrecanase-generated aggrecan catabolites, using media from control cultures and those exposed to either OSM or RA. Interestingly, there was evidence of low levels of aggrecanase activity in the control cultures (FIGURE 2), which was in contrast with observations in control cultures of bovine or porcine articular cartilage (results not shown). However, exposure to either OSM or RA caused a marked increase in aggrecanase activity (FIGURE 2 +). Based upon these findings, we decided to use OSM as the catabolic stimulants for our studies investigating the effects of n-3 PUFAs on canine articular cartilage degradation.

FIGURE 3 shows the results of GAGs released from canine articular cartilage (n = four different dogs) previously cultured in the absence or the presence of the n-3 PUFAs ALA, EPA or DHA (at 100 or 300 µg/ml) then subsequently cultured for four days with or without OSM. There were no significant increases in GAG release into the media of control cultures exposed to either doses of ALA, EPA or DHA (FIGURE 3). Exposure to OSM caused a significant increase in GAGs released into the medium. Interestingly, prior exposure of the cartilage explants to ALA caused an even further increase in GAG release, whilst exposure to DHA showed no significant increases. However, in marked contrast to the findings with ALA and DHA, prior exposure of the cartilage explants to ***EPA caused significant dose dependent decreases in GAG release*** when the explants were exposed to OSM. Western blot analyses, using mAb BC-3, indicated that the OSM-induced aggrecanase activity was also reduced by exposure to EPA (but not ALA or DHA) in a dose dependent manner (results not shown). In this initial study, however, we observed some variation in the extent of this EPA abrogation of proteoglycan degradation. This variation may have been caused by differences in age, breed or diet of the dogs used in these studies. We therefore

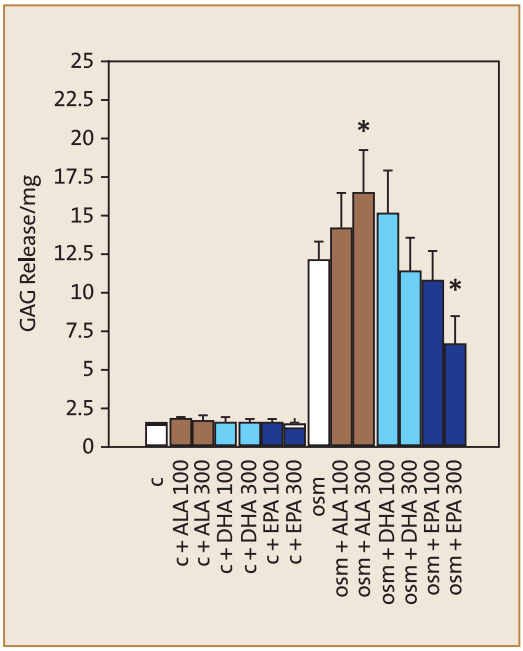

Figure 3. Proteoglycan (GAG) release into the culture medium of canine articular cartilage explant cultures (n = four dogs) that were exposed to PUFAs prior to being cultured either in serum free media (c) or in the presence of OSM.
PUFAs used in these experiments were ALA, DHA and EPA, used at concentrations of 100 & 300 mg/ml.

undertook an additional study, in which we obtained articular cartilage from five dogs of the same breed, of similar age (12-14 years old), that had been fed the same diet for the past two years.

FIGURE 4 shows the GAG release obtained from canine articular cartilage explants from the non-overtly pathological knees of the five older beagle dogs (same breed, age and diet). In this study, only

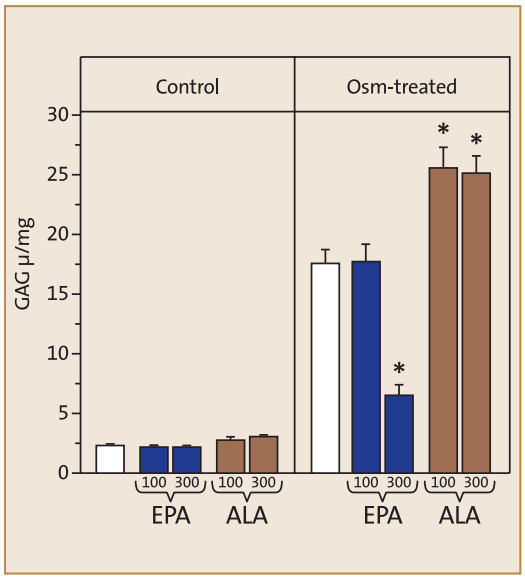

Figure 4. Example of data obtained for proteoglycan (GAG) release into the media of one of the five beagle dogs (same age and feeding regimen).
Canine articular cartilage explant cultures were exposed to EPA and ALA before being cultured a further four days in the presence of serum-free media without (Control) or with Oncostatin M (Osm-treated). All five dogs showed very similar GAG release profiles.

ALA and EPA were tested and the catabolic stimulant used to induce aggrecan catabolism was OSM. As seen in our previous study (FIGURE 3), prior exposure to either ALA or EPA had no effect on the level of GAG released into control cultures (FIGURE 4). Similarly, exposure to OSM caused a very significant increase in GAG released into the media and exposure to ALA further increased these levels in all five dogs (FIGURE 4). Importantly, the level of GAG released into the media of all five dogs (including the pathological right knee of the fifth dog) that had been cultured with EPA prior to their exposure to OSM all showed a dose-dependent decrease in GAG release (FIGURE 4). Western blot analyses using mAb BC-3 (FIGURE 5) also demonstrated a dose-dependent abrogation of aggrecanase activity that paralleled this decrease in GAG release.

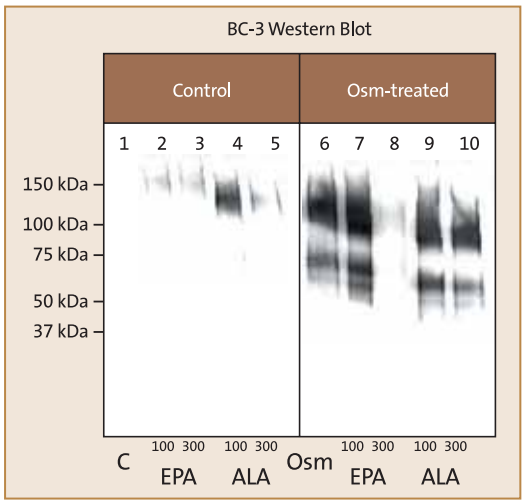

Figure 5. Western blot analyses of the samples illustrated in FIGURE 4 using monoclonal antibody BC-3 that recognises aggrecanase-generated neo-epitopes on cartilage aggrecan catabolites.
Samples from the different columns shown in FIGURE 4 were loaded into the electrophoresis lanes on an equal GAG basis; i.e. 10 mg GAG per well. Lane 1: control (C) with no PUFA pre-culture; Lanes 2 & 3 (EPA) and 4 & 5 (ALA) pre-culture at 100 & 300mg/ml to control cultures; Lane 6: oncostatin M (Osm) exposed with no PUFA pre-culture; Lanes 7 & 8 (EPA) and 9 & 10 (ALA) pre-culture at 100 & 300mg/ml prior to their exposure to Osm.

DISCUSSION

Our results indicate that hyaline articular cartilage obtained from different animal species respond very differently to a variety of catabolic stimulants mimic mechanisms of cartilage degradation in the pathogenesis of degenerative joint diseases. In this study, we demonstrated that exposure to either OSM or RA is needed to cause significant increases in proteoglycan / aggrecan degradation (increased GAG release) when using canine articular cartilage explant culture systems. These findings are in contrast with those found when bovine, porcine or human articular cartilage explant culture systems were used.

We decided to investigate the effects of n-3 PUFAs in these *in vitro* models of cartilage degradation using canine articular cartilage explant cultures that had been exposed to OSM. Our results indicated that prior exposure to ALA caused a further increase in proteoglycan degradation over that seen with OSM alone. Interestingly, prior exposure to DHA showed no effects on GAG release. In marked contrast to ALA and DHA, prior exposure of the canine cartilage explants to the PUFA EPA demonstrated a dose-dependent reduction in proteoglycan catabolism that was paralleled by the abrogation of aggrecanase activity. However, we noted some variability in these EPA-mediated effects between the four different breeds of dog of unknown age. We therefore decided to repeat these experiments using cartilage obtained from five dogs of the same breed and very similar older age, which had been fed with the same diet. This more controlled study confirmed the findings and conclusions extrapolated from our initial study, indicating that only EPA exposure was effective in abrogating cartilage degradation. The reason for this selective effect of different n-3 PUFAs in canine cartilage is not clear.

CONCLUSION

In summary, our data provide evidence that there are differences between animal species in the way the hyaline articular cartilage behaves in '*in vitro*' culture systems that use different catabolic stimulants to induce cartilage proteoglycan (and possibly collagen) degradation. These studies suggest that the use of OSM or RA generates greater levels of cartilage proteoglycan catabolism when canine articular cartilage is used in the explant culture systems. Our data also indicate that EPA, and not DHA or ALA, is the most appropriate n-3 PUFA to abrogate the aggrecanase-mediated cartilage proteoglycan catabolism in these in vitro models of cartilage degradation. These studies suggest that dietary supplementation of dog food with EPA may prove to be efficacious in slowing down the rate of cartilage degradation in canine degenerative joint diseases.

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Myeloperoxidase, a Highly Sensitive and Specific Pain Indicator in Osteoarthritis

INTRODUCTION

In Germany 20 per cent of all dogs suffer from a painful disease of the locomotor apparatus, 13 per cent suffer from Osteoarthritis (OA).[1] OA can be primary, without underlying initiating cause, or secondary to joint diseases such as hip dysplasia, patella luxation, osteochondritis dissecans or trauma, including intra-articular fractures and rupture of the cranial cruciate ligament.[2,3,4] Examination of the lame dog includes clinical orthopaedic and radiographic examination, but radiography is not very useful in the early stages of the disease process. The composition of the synovial fluid changes during development of OA; detecting such changes might become more important for early diagnosis of OA in the future.[5]

In a randomised study, we measured the myeloperoxidase (MPO) level in the synovial fluid of dogs seen at the small animal clinic of the Free University of Berlin. MPO is a highly reactive enzyme produced by phagocytic cells; it reaches the synovial fluid after degranulation of neutrophils and via active secretion.[6,7,8] MPO catalyses the reaction of hydrogen peroxide and chloride ions to hypochloride acid, which is even more invasive than hydrogen peroxide itself altering synovial fluid and damaging cartilage.[9,10,11,12] MPO is used in human medicine to diagnose rheumatoid arthritis, and could be an appropriate marker of changes in diarthrodial joints in dogs.[12]

MATERIAL AND METHODS

In this study, 104 dogs were selected from patients that underwent surgery at the small animal clinic of the Free University Berlin for lameness of orthopaedic origin, and were randomised (TABLE 1). The dogs were divided into groups according to aetiology: ruptured cranial cruciate ligament (RCCL), fragmented coronoid process (FCP), coxarthrosis following hip dysplasia (HD), osteochondritis dissecans (OCD), and a mixed group of different disorders (mixed). Dogs that

had received any antiphlogistic drugs or had less than 0.8 ml synovial fluid in the joint were excluded from the study. Eight dogs euthanased for other reasons and without clinical or radiological signs of musculoskeletal disease were included in the study as a control group. These dogs were of different breeds, gender, age and size.

Collection and evaluation of the synovial fluid

Animals were anaesthetised and, after radiographic examination, the joint was punctured before the capsule was incised and synovial fluid aspirated under sterile conditions. Synovial fluid was then examined for viscosity, total protein (TP), specific gravity (SG), complete cell count and MPO content.

The viscosity was determined by measuring the length of the synovial fluid droplet as it leaves the tip of a needle. Cell counts were performed as described by Sawyer. [13] SG and TP were measured with a refractometer (Fa. Krüss, Mod. HRM 18).

The MPO content was determined at the Laboratory of the Institute of Physiological Chemistry of the Phillips University Marburg. It was measured in the cell-free supernatant fluid of the synovial fluid using an o-dianisidine assay. Each analysis was performed twice and the average calculated.

Radiographs were evaluated according to methods decribed by Brunnberg (stifle joint), Brunnberg and Viehmann (elbow), and by Morgan *et al*., Carrig and Martinez (shoulder). [14-18] The radiographic changes were ranked on a scale from zero to four, with zero being normal and four being severely affected (TABLE 2).

During surgery the joints were visually examined and divided into three categories according to the changes seen on cartilage, bone and capsule. Statistical analysis was performed with SPSS for Windows, release 6.1. Patient groups were compared using the Man-Whitney U-Tests.

Correlations between MPO activity and other parameters such as radiographic findings, changes in the synovial fluid and the surgical findings were evaluated applying different statistical methods.

RESULTS

Classification of the dogs according to body weight suggested that heavier dogs are more likely to be affected by orthopaedic diseases needing surgery (TABLE 1); but there was no influence of gender.

TABLE 1

	n	Average body weight (kg)	Average age (months)
RCCL	56	36.6	57.3
FCP	16	34.5	13.0
HD	11	33.2	22.8
OCD	11		9.2
Mixed	10	32.7	40.4
Control	8	33.5	55.6

Table 1. Body weight and age of the dogs with different disorders.

Analysis of the synovial fluid

The complete cell count was higher in all affected joints than in the control group. In all joints the synovial fluid viscosity was reduced to a variable degree compared with controls, except for 5/11 OCD joints, 5/24 joints with FCP and 1/56 joints with RCCL. TP was significantly increased in all affected joints except for the OCD cases (TABLE 3), and there was a significant difference in SG between affected joints and controls. The MPO content was at least twice as high in affected joints as in healthy joints and the difference was statistically significant (FIGURE 1). Dogs of the mixed patient group were not included in the statistical analysis because of the small number of cases.

Radiographical findings and findings during surgery

All affected joints showed radiographic changes, with the exception of the dogs with RCCL and 7/24 joints with FCP. During surgery, no lesions of osteoarthritis were found in the dogs with RCCL, whilst the others showed changes in at least one of the articular structures.

TABLE 2

Score		Severity of radiographic features
0	normal	-
1	doubtful	minimal osteophytosis and possible bone cyst formation
2	minimal	obvious osteophytes and possible bone cysts
3	moderate	multiple moderate osteophytes and some subchondral sclerosis with deformity of bone ends
4	severe	large osteophytes, severe sclerosis and obvious deformity of bone ends

Table 2. Radiographic criteria of OA.

TABLE 3

	Normal	Osteoarthritis
Volume	0.1 - 1 mL	↑ or ↓
Colour	Colourless to yellow	Colourless to yellow
Turbidity	Transparent	Transparent or minimal turbidity
Viscosity	High	↓
Protein concentration	2.0 - 2.5 g/dL	3.0 – 4.5 g/dL
Nucleated cells	< 1000/µL	1000 – 5000/µL
Mononuclear cell	95%	> 90%
Neutrophils	≤ 5%	< 10%

Table 3. Analysis of synovial fluid.

DISCUSSION

In our study, the average cell count in synovial fluid was 522 cells/µL, the highest value being 875 cells/µL. Although, the cell count was always higher in the affected joints than in the controls, all values were below what is cited in literature as the maximum cell count in healthy joints, which range from <3000 cells/µL, over <1500cells/µL to < 1000 cells/µL. [13, 19, 20]

Although the evaluation of synovial viscosity is subjective, it gives useful information; a droplet length of at least 5 cm is generally accepted as the norm for healthy joints. [19, 21, 22] It is widely accepted that OA does not alter synovial fluid viscosity, in our study however, viscosity was unchanged in only 13/120 joints, whereas all other joints showed a variable degree of change. [19, 21, 22]

The average TP in our study varied between 2.7 g/dL and 4.0 g/dL, whilst reference values for healthy joints are reported to be between 1.9 and 3.5 g/dL. [23, 24]

SG of the synovial fluid varied between 1012 and 1021 in the control dogs, and between 1023 and 1029 in affected joints, SG in the control dogs was within the normal range (SG = 1015-1017) published by Farham. [25]

Radiographic evidence of OA was present in all but 10 joints; half of which were in the FCP group. The fact that the diagnosis of FCP was based on clinical findings rather than on radiography might explain this. [15]

In 10/56 joints of the RCCL group no signs of degenerative joint disease were seen during surgery. None of the markers for OA that have been studied so far including IL-1, TNFα, stromelysin, and collagenase, can be used as a marker for severity of OA. [26-33]

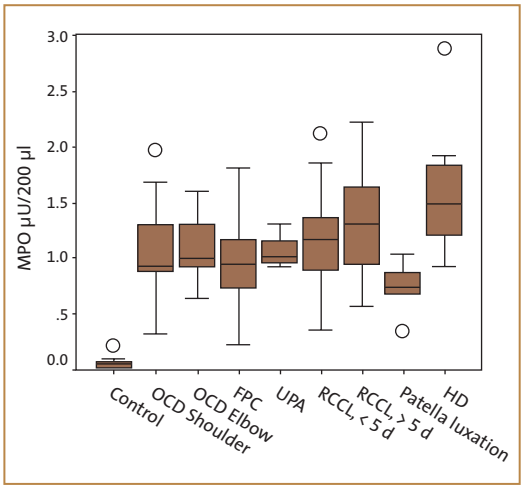

FIGURE 1. MPO activity in relation to the different aetiologies of joint diseases.

Myeloperoxidase

The average MPO activity found in healthy joints was 0.07 µU/200µL (0.02 – 0.23 µU/µL) with a median activity of 0.05µU/200µL (FIGURE 1). The average MPO activity in the affected joints was 25 to 40 times higher than in the controls, with the lowest value being twice that of the control. This confirms data published by Schiller *et al*. showing increased MPO activity in cell culture and in synovial fluid of humans with rheumatoid and immune-mediated arthritis. [12] As in human patients, in dogs we didn't find a correlation between MPO activity and radiographic or surgical findings (Kruskal-Wallis-Test) or changes in synovial fluid i.e. SG, TP, or complete cell count. [34] We did, however, find a

TABLE 4

Viscosity	MPO activity Mean (range) µU/200µL
Normal	0.59 (0.02-1,68)
Decreased	0.84 (0.03-2.16)
Severely decreased	1.29 (0.35-2.23)
Watery	1.31 (0.58-2.88)

Table 4. Correlation between synovial fluid viscosity and MPO activity.

significant correlation between MPO activity and viscosity of the synovial fluid (TABLE 4). The mean MPO activity in low viscosity synovial fluid was almost double of that in normal viscosity synovial fluid. This is logical since MPO favours the formation of HOCl, which splits hyaluronan in the synovial fluid, making it more fluid (FIGURE 2). [35] This was confirmed by Green *et al*. who were able to decrease hyaluronan viscosity significantly by adding MPO. [10]

CONCLUSION

There is no correlation between radiographic evaluation, observations made during surgery, and changes seen in the synovial fluid. MPO has never been studied in the canine joints, but is used as a marker for immune-mediated OA in people. Within the synovial fluid a good correlation was found between viscosity and MPO activity. An increase in synovial MPO activity can be considered highly specific for OA in dogs.

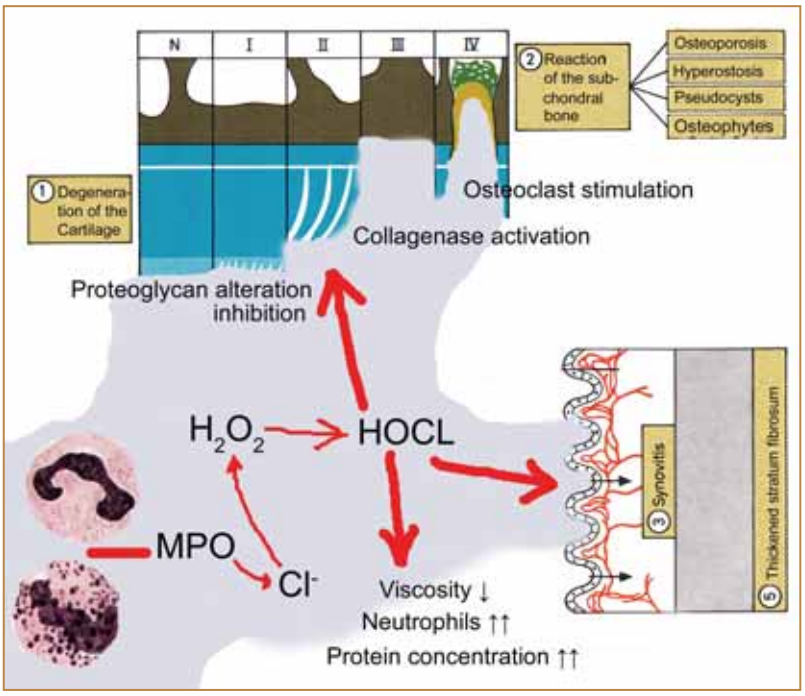

Figure 2. Role of MPO and HOCL in the pathogenesis of joint diseases.

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Lipids and Osteoarthritis in Human Joints

INTRODUCTION

Osteoarthritis (OA) is the most common joint disorder in the UK and affects more than one million individuals. It is strongly associated with ageing and is a major cause of pain and disability [Arthritis Research Campaign (***arc***, 1998)]. Although strongly associated with age, OA is not an inevitable consequence of ageing and the two processes are quite distinct. [1] Primary OA is a heterogeneous group of conditions that can affect most of the synovial joints and its aetiology is largely unknown, though various risk factors have been identified. Primary OA may be classified as localised, affecting a single joint, or generalised, affecting three or more joint groups. [2,3] Radiographic changes are found in more individuals than are symptomatic and many patients are symptomatic in one joint only; the most common joints being hands, knees and hips. Knee OA is the most prevalent, affecting 10-25 per cent of the population over 65 years, particularly women (***arc***, 1998). In the UK five per cent of the population over the age of 60 have a hip replacement. [4] The most widely used epidemiological criteria for OA are based on specific radiological changes; joint space narrowing, subchondral bone sclerosis, subchondral cyst formation and marginal osteophytes. [5,6] It is notable that three of these four are related to bone despite the traditional emphasis on the articular cartilage.

RISK FACTORS AND PROGRESSION OF OSTEOARTHRITIS

Age and gender

Important risk factors for primary OA include age, sex, family history, genetics, obesity, nutrition and bone density. OA is not an inevitable consequence of ageing and the prevalence of OA before the age of 55 is roughly equal between the sexes. [1] In older age groups, females are affected disproportionately with frequent multiple joint involvement. It is thought there could be a hormonal mediation of OA as pre-menopausal women seem to be protected against knee OA and oestrogen has been shown to be protective, whilst testosterone and progesterone appear to enhance OA. [7,8] Studies of family history, extended family

correlations, mother-daughter studies and twins have all emphasized the heritability of OA [for REVIEW, see Holderbaum *et al*, 1999 (Ref. 9)].

Links with obesity

OA and osteoporosis (OP) are the two most significant musculoskeletal causes of ill-health, and even death, in the increasingly elderly western population. Bone is badly affected in both diseases (FIGURE 1). In OP, a loss of bone results in fragility and increased risk of fracture. In OA, there is a proliferation of poorly mineralised bone. [10]

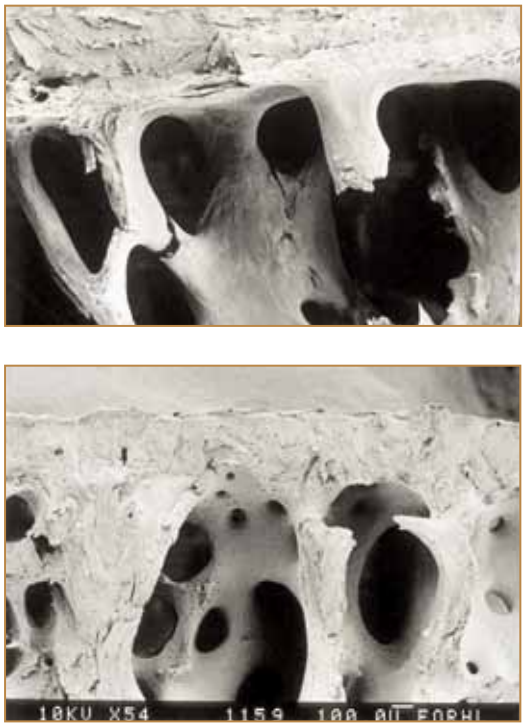

Figure 1. Scanning electron micrographs of osteoporotic (top) and osteoarthritic (bottom) bone from human femoral heads.
A loss of bone in OP is in contrast to the proliferation of woven bone and evidence of considerable osteoclastic activity in OA.

In parallel with these changes in the bone, patients with OP generally have a below bone mineral density (BMD), whilst OA patients have an increased BMD. OP fractures in OA patients are seen occasionally, but only on very old patients, suggesting OA may have a protective effect on the progression of OP. [11]

Until recently, it was uncertain whether obesity was an actual cause of OA, or whether it was just a morbid consequence of the relative immobility and disability imposed by the disorder. If it was found to be causal, was there a metabolic component or simply a problem of mechanical overloading? Several studies over the past few years have established that being overweight predisposes to the development of the knee OA, specifically in the tibiofemoral and patellofemoral compartments, and seems to be more common in females. [7, 12-14] An observational study, the Framingham Study, found obesity preceded and was associated with the appearance of radiological knee OA 30 years later, leading to their suggestion that mechanical factors played a significant role in the progression of OA. The Framingham study also showed that overweight people, who then lost weight, significantly reduced their risk for OA. [15] There are no consistent associations for the hip but a recent review indicates a moderate association between hip OA and obesity. [16] Having proved that obesity increases susceptibility to OA, the current point of contention is whether the involvement of obesity is purely mechanical or, alternatively, whether there is a metabolic contribution. Whilst mechanical overloading, especially of hips and knees may be a factor, links between hand OA and obesity are harder to explain and suggest that metabolic factors could be responsible. Such an association could also explain the changes in bone at the iliac crest, remote from joint load bearing, found in patients with hand OA. [17-19]

OSTEOARTHRITIS: A SYSTEMIC DISORDER?

Epidemiologists have suggested a systemic component to generalised OA. There are not only changes in cartilage and bone, as described above, but also hypertrophy of ligaments and the joint capsule, muscle weakness and an increase in adiposity (i.e. overweight). A hypothesis has been proposed by Aspden, Scheven and Hutchison here at the University of Aberdeen to try to draw together these many skeletal changes. [20] They hypothesised that primary generalised osteoarthritis is a systemic disorder involving mesenchymal stem cell differentiation and lipid metabolism. Osteoblasts and chondrocytes (as well as myocytes and fibroblasts) share a common mesenchymal stem cell precursor with adipocytes. They can be stimulated in vitro towards a more adipocytic phenotype by altering the culture environment. Hence, systemic factors, such as fatty acids, could, at least partly, be responsible for the changes seen in primary OA, both in the joint space and in distant tissues, by altering the direction of mesenchymal stem cell differentiation and the proliferation of

cells. Elevated levels of serum cholesterol have also been reported and an increased risk of coronary heart disease, again indicating that systemic factors may involved. [21-23]

MESENCHYMAL STEM CELL DIFFERENTIATION

Bone marrow contains certain multi-potent, stem-like cells called mesenchymal stem cells (MSCs). These cells can differentiate into a variety of cell types, including osteoblasts, chondrocytes and adipocytes, as shown in FIGURE 2. Studies *in vitro* using MSC cultures have shown that these cells can be pushed along these different pathways by altering the culture environment. [24] Furthermore, Diascro *et al*. demonstrated that the addition of rabbit serum (RS), which is high in fatty acids, to pre-osteoblasts from human osteosarcoma SaOS-2/B10 and MG-63 cell lines resulted in a change to an adipocytic-like phenotype. [25] Linoleic acid seemed to drive the cells to an adipocytic phenotype, as the amount present in RS was four times higher than in foetal bovine serum (FBS). Similarly, studies such as those performed by Lippiello et., have documented an increase in lipids in articular cartilage from patients with OA, compared with tissue from similarly aged, non-diseased patients. [26]

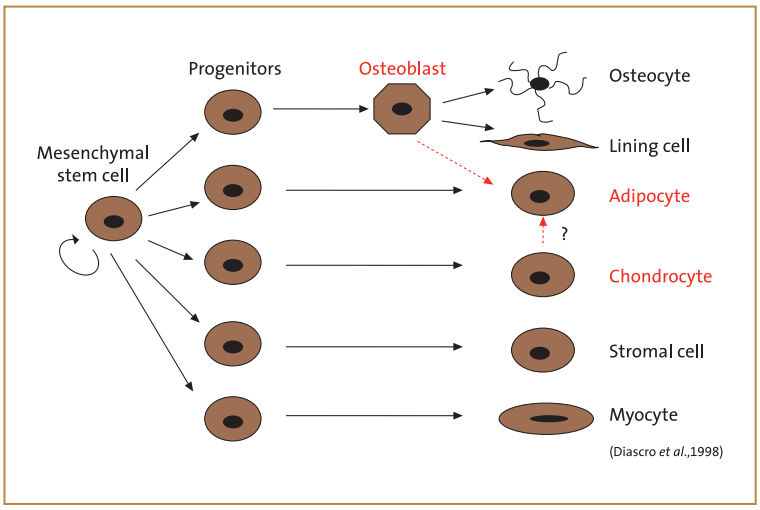

Figure 2. Schematic diagram showing mesenchymal stem cell differentiation.
The dotted-line arrows represent the theoretical change in osteoblastic and chondrocytic phenotypes seen in OA. (Diascro et al. 1998) [25].

Taking this evidence and applying it to the hypothesis proposed by Aspden *et al*., the loss of cartilage and abnormal bony remodelling seen in OA may be due to a change in osteoblastic and chondrocytic phenotypes towards a more adipocytic cell characteristic. [20] This could result in an impaired ability to maintain homeostasis within these tissues and it is thought that signalling molecules, such as fatty acids, could stimulate this alteration.

FATTY ACID COMPOSITION OF OA AND OP FEMORAL HEADS

Given the above hypothesis, we asked the question as to whether there are differences in the lipid content and fatty acid composition of the bone marrow in OA and OP. In OP there are fewer osteogenic cells and a greater number of adipocytes in the marrow and it has been proposed that a switch from osteoblastic to adipocytic phenotypes could explain the loss of bone. [27] Structurally, OP bone has few and narrow trabeculae with large spaces between them. In OA the trabeculae are thicker and so have less space between them. The greater porosity in OP combined with a change in tissue types suggests that one might expect to find a higher fat content in OP cancellous bone. However, anecdotal evidence suggested an increased amount of fat in OA bone: in the laboratory fat globules are often found floating in the cell culture medium, and during surgery fat is commonly expressed from the bone during resection of the tissue. To try to resolve this we have measured the fat content of the bone from the femoral heads of patients with either OP or OA.

Lipid content was measured from five femoral heads from patients undergoing total hip replacement for OA and five from osteoporotic fracture of the femoral neck. The bone was chopped up and powdered in a liquid nitrogen freezer mill (Model 6750, Glen Creston Ltd, Middlesex, England). The lipid was extracted using a standard chloroform-methanol extraction. Lipid was weighed and the mass of lipid per unit mass and unit volume of bone was calculated. Fatty acid methyl esters (FAME) were prepared from the extracted lipid by transmethylation using 0.5 M sodium methoxide as described by Christie (1982) with slight modifications. Samples and standards were injected onto a Varian 3800 Gas Chromatograph fitted with a J&W Scientific Column, DB-225 and analyzed by running for 40 minutes to determine the respective fatty acid profiles.

The mass of lipid per unit mass of tissue, was found to be 0.24 ± 0.04 g/g (mean ± sd, *n*=5) in the OA group and 0.21 ± 0.05 g/g in the OP group. Bone mass was taken to be tissue mass minus lipid mass, and the lipid-to-bone ratios became 0.31 ± 0.07 g/g (OA) and 0.27 ± 0.07 g/g (OP). However, the proliferation of bone in OA and the loss in OP means that the apparent density of OA bone is considerably greater (0.71 g cm^{-3}) than OP bone (0.38 g cm^{-3}) so the fractional volume available for the fat is considerably smaller; porosity 59% in OA, 80% in OP.[10] Assuming that the bone defines the total tissue volume, this volume was found by dividing the mass of the bone by the apparent density. The amount of lipid per unit volume of tissue then became 0.22 ± 0.05 g cm^{-3} in OA and 0.10 ± 0.02 g cm^{-3} in OP (*P* = 0.002, t-test).

There were also a number of differences in the fatty acid composition of the extracted lipids between OA and OP. Those that were significant (Student's t-test) are shown in TABLE 1. The saturated stearic acid (C18:0) was lower in OA than OP bone, but the omega-6 (*n*-6) fatty acids were higher by between 50-90%.

Despite the greater marrow space available in OP bone, this study found that the amount of fat in a given volume of OA cancellous bone tissue is approximately twice that found in the same volume of OP bone. There are also significant differences in the fat composition in terms of fractional amounts of specific fatty acids between the two diseases. It is notable that all the fatty acids significantly increased in OA are of the omega-6 (*n*-6) series, which are precursors of the most pro-inflammatory eicosanoids. Of particular note is arachidonic acid, the precursor of prostaglandin E2. Arachidonic acid is reported to be increased in cartilage, serum and synovial fluid of OA patients and our study shows it to be present in almost twice the concentration in OA bone compared with OP bone.[26]

TABLE 1

Fatty acid	OP (Mass %)	OA (Mass %)	*P*
C16:1 Palmitoleic	4.1 ± 1.1	7.0 ± 1.2	0.005
C18:0 Stearic	4.67 ± 0.30	3.26 ± 0.47	<0.001
C20:2n-6 Eicosadienoic	0.102 ± 0.023	0.151 ± 0.023	0.011
C20:4n-6 Arachidonic acid	0.245 ± 0.029	0.479 ± 0.067	<0.001
C20:3n-6 Dihomo gamma-linolenic acid	0.117 ± 0.028	0.166 ± 0.019	0.012
C22:4n-6 Docosatetraenoic	0.119 ± 0.017	0.178 ± 0.020	0.001

Table 1. Fatty acid composition of lipids extracted from bone and marrow.
The fatty acid composition differed between osteoarthritic and osteoporotic patients, expressed as a percentage of total fatty acid mass (mean ± sd). Significance values were obtained using Student's t-test.

CONCLUSION

We have hypothesised that primary generalised osteoarthritis (OA) may be a systemic disorder affecting the whole musculoskeletal system and involving altered lipid metabolism.[28] The proliferation of bone and fat in OA points towards lipids playing a significant role in the pathogenesis of OA and the increased levels of (*n*-6) fatty acids suggest there may be an inflammatory component, albeit perhaps, requiring a broader interpretation of inflammation. It may also provide part of the key to understanding why OA and OP appear to lie at opposite ends of the spectrum of bone masses, though the mechanisms mediating this are still unclear.

ACKNOWLEDGEMENTS

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Comparison of Imaging Techniques used in the Osteoarthritic Joint and their Interpretation

INTRODUCTION

Osteoarthritis (OA), the most common type of joint disease affecting humans and animals, is a heterogeneous group of conditions resulting in common histopathological and radiological changes. It is a degenerative disorder resulting from the biochemical breakdown of articular cartilage in the synovial joints. Common synonyms for osteoarthritis include osteoarthrosis and degenerative joint disease. Although the term 'osteoarthritis' is often used, 'osteoarthrosis' may be more appropriate. Degenerative changes are the predominant factor contributing to disability. The exact aetiology of osteoarthritis is unknown. Multiple factors, such as genetics, trauma, and obesity, interact to cause this disorder. Any event that changes the environment of the chondrocyte has the potential to cause osteoarthritis. Although usually occurring as a primary disorder, osteoarthritis can be secondary to other disorders. The diagnosis is largely clinical because radiographic findings do not always correlate with symptoms.

In people, genetics play a role in the development of OA, some people may be born with defective cartilage or with slight defects in joint congruity. As a person ages, these defects may cause early cartilage breakdown. In the process of cartilage breakdown, there may be some inflammation, with release of enzymes and further cartilage damage.

The joint structures involved in the morphogenesis of OA include the cartilage, subchondral bone and synovial membrane.

IMAGING

The history of musculoskeletal imaging begins with Roentgen's discovery of X-rays in 1895. Plain radiography has since become the first and often the only imaging technique used for diagnosis and follow up of joint abnormalities. In veterinary orthopaedics, plain radiography has been the routine imaging technique for decades, and is still routinely used in most practices. In human orthopaedics, other imaging techniques such as arthrography, scintigraphy,

computerised tomography (CT), magnetic resonance imaging (MRI), ultrasound (US) and arthroscopy are used on a daily basis.

Conventional radiography

Conventional radiography is an excellent imaging technique for bony structures but is a poor method for visualising soft tissue structures. It provides a greater spatial resolution than either MRI or CT. The disadvantage, however, is that the two-dimensional display of three-dimensional structures results in superimposition, which can mask important changes. Plain radiographs usually can confirm the diagnosis of osteoarthritis, although the findings are non-specific, i.e. absence of radiographic changes does not exclude the presence of osteoarthritis. Moreover, many patients (people and animals) with radiographic changes consistent with osteoarthritis are asymptomatic, suggesting that the presence of radiographic changes in the absence of clinical signs should not always be interpreted as pathological.

Details that can be derived from plain radiographs include information on the size, contour, density, and location of changes that are present in or around a joint. Areas that can be evaluated include the subchondral bone plate, trabecular subchondral bone, articular margins, and areas where ligaments, tendons, and the joint capsule attach. Specific changes in the appearance of osteoarthritic joints include narrowing or ablation of the joint space, increased density of the subchondral bone (eburnation), new bone formation of joint margins (osteophytosis) (FIGURE 1), joint deformity with preservation of articular margins, proliferative and lytic changes at the attachment sites of the joint capsule and the supporting ligaments, and partial to complete ankylosis .

The severity of new bone formation is often underestimated on conventional radiography and can better be evaluated on CT (FIGURE 2).

In people and horses joint space narrowing has been a well-accepted indicator of articular cartilage degeneration and is considered as a cardinal radiographic feature of the disease. In small animals, the loss of joint space is not a reliable sign as only non-weight-bearing radiographs are used. An indirect method for evaluating the status of the articular cartilage in canine shoulder joints with osteochondritis dissecans has been reported and is based on the size of the lesion in the subchondral bone and the presence of the vacuum phenomenon (FIGURES 2 & 3).

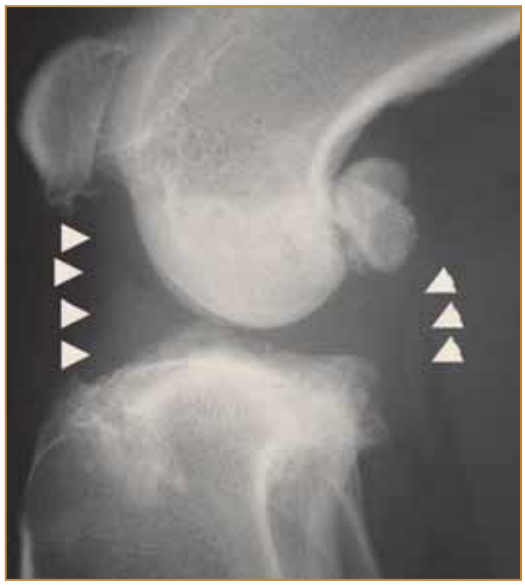

Figure 1. Arthritic stifle of a dog.
Peri-articular new bone formation is visible as well as joint effusion (arrow heads).

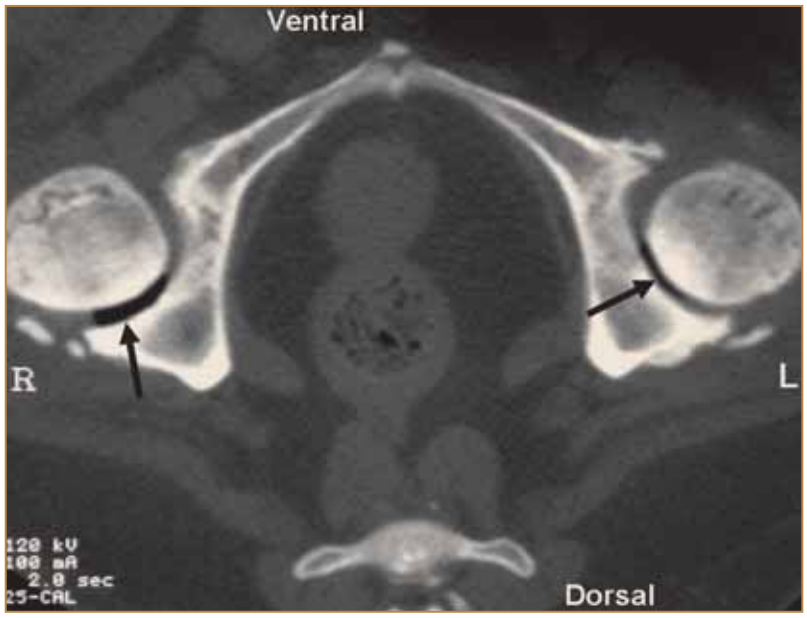

Figure 2. CT image of the hip joints of a dog with arthritic changes.
New bone formation, subchondral sclerosis and a vacuum phenomenon (arrows) are present in both joints.

Individual soft tissue structures are not visualised as easily as the bony structures unless they are bordered by fat (e.g. in facial planes or in the cranial aspect of the stifle) (FIGURE 1). Because of these limitations, early manifestations of OA are difficult to visualise by conventional radiography.

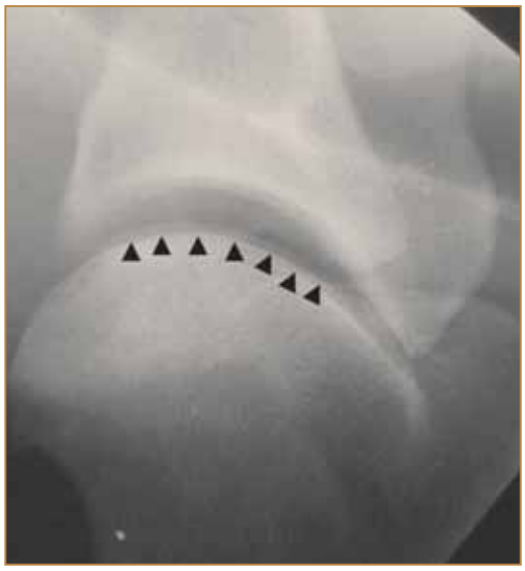

Figure 3. Arthrotic shoulder joint of a dog.
A vacuum phenomenon, a sign of cartilage degeneration is visible (arrow heads).

Arthrography

Arthrography is seldom used in small animal orthopaedics but is an interesting and simple technique readily available to most veterinarians. Although probably not as accurate as the newer imaging techniques (arthroscopy, MRI, and ultrasound), it provides information on intra-articular structures not seen on survey radiographs. Within the shoulder joint, arthrograms can roughly visualise the following structures: the articular cartilage, intra-articular ligaments, the extent of the joint capsule and the synovial surface outline (FIGURE 4). To do so, one to four millilitres of preferably a non-ionic, low-osmolar contrast medium has to be injected intra-articularly. Exposures should be made within five minutes after the injection, because the contrast medium is rapidly absorbed by the synovial membrane. The use of contrast medium allows to demonstrating cartilage fissuring and fragmentation such as occurs in osteochondritis dissecans, as contrast material infiltrates underneath the articular cartilage. In this way a distinction can be made between clinical and non-clinical lesions. Synovial proliferation may appear as a thick, irregular synovial outline or as a small filling defect within the joint capsule (FIGURE 5).

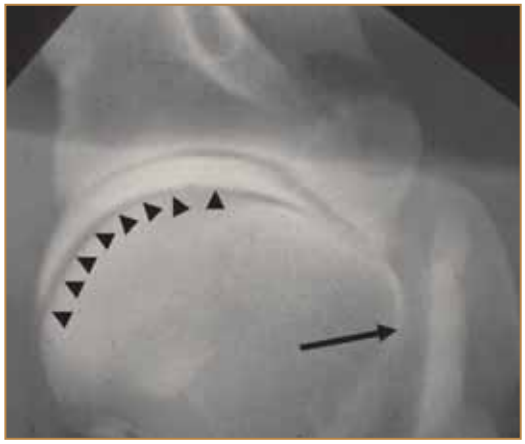

Figure 4. Arthrogram of a normal canine shoulder.
The articular cartilage (arrow heads), the joint capsule and the biceps tendon (arrow) are visible.

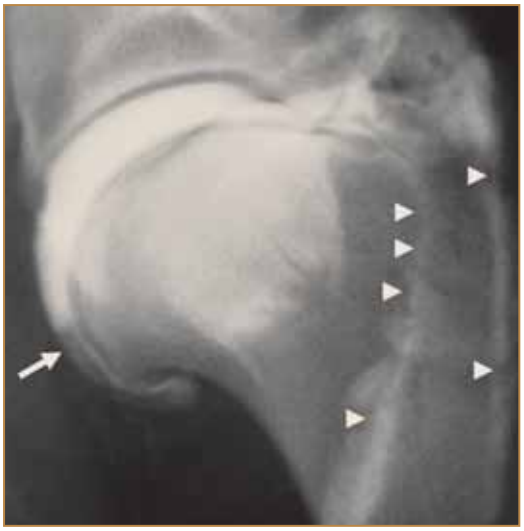

Figure 5. Arthrogram of a dog's shoulder with signs of arthritis and synovitis.
Slight joint effusion (arrow) and irregular synovial outline of the bicipital tendon sheath are visible (arrow heads).

High-definition micro-focal radiography

Magnification radiographs can be made by increasing the distance between object and film while maintaining the same x-ray tube-film distance. Recent advances in technology have lead to the development of a high-definition micro-focal X-ray unit permitting macro-radiographic examination of joints at five to ten fold magnifications and with high spatial resolution. This technique allows for detection of early, subtle changes associated with OA. Magnification radiography can also be combined with arthrography, a technique, called macro-arthrography, by which cartilage thickness can be measured and show possible cartilage thinning.

By decreasing the air gap between the object and the film the amount of scatter radiation is reduced. Therefore, magnification radiography provides greater sharpness, reduced noise, and better contrast than conventional radiography. However,

this magnification technique requires the use of a small focal spot in the X-ray tube, whereas most X-ray machines routinely used in practice have a focal spot larger than one millimetre, making them unsuitable for magnification radiography (FIGURE 6).

Scintigraphy

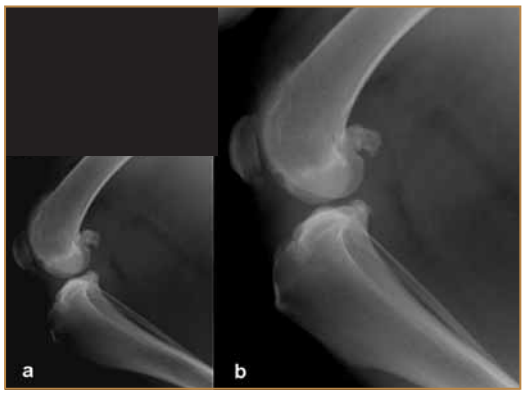

Figure 6a + b. Classical radiograph (a) and magnification (b) of an arthrotic stifle of a dog.
This magnification was performed using a focus of 0.3 mm and shows more bony details in high resolution.

Skeletal scintigraphy is a commonly performed imaging procedure in veterinary medicine, mainly in large animal orthopaedics. Whilst conventional radiography is an excellent method to investigate morphologic changes in bones, radionuclide techniques provide information about the metabolic function of the skeleton. Scintigraphy is a useful technique for surveying the entire skeleton, to localise the cause of obscure lameness, or to be used in case of uncertain radiographic findings (FIGURE 7). Skeletal scintigraphy is a very sensitive method allowing for early disease detection, though not very specific. Radiography, on the other hand, is less sensitive but more specific. Also the spatial resolution offered by scintigraphy is not good enough to specify anatomic structures (FIGURE 7). In some instances it is difficult to determine whether a difference in counts between two joints represents a meaningful finding. Comparison of bilateral images, acquired at the same time, and quantitative analysis of joint images by computer can provide diagnostic guidelines.

Fluorodeoxyglucose positron emission tomography (FDG) PET has proved to possess potential in imaging cartilage lesions. PET's future lies in image fusion with MRI and CT.

Computerised tomography

CT has several advantages over plain radiography. It is a cross-sectional imaging technique using X-rays and computers. Better soft tissue differentiation and absence of superimposition are the major advantages of CT over conventional x-ray techniques. CT scanning enables a more detailed and specific morphological diagnosis than radiography. CT greatly facilitates examining complex joint structures such as the elbow and tarsus. Another advantage is that transverse CT images can be reformatted in multiple anatomic planes. Although the spatial resolution of CT images is poorer than that of classical film-screen radiography, the cross-sectional image display and the superior discrimination of tissue attenuation offered by CT enables differentiation of soft tissue structures that can not be perceived on conventional radiographs (FIGURE 8). Subtle new bone formation and bone lysis are better identified on CT images when compared with conventional radiography, because of their better physical density discrimination, the ability to manipulate the grey scale of the digital image, and the elimination of overlying structures. Degenerative changes can be

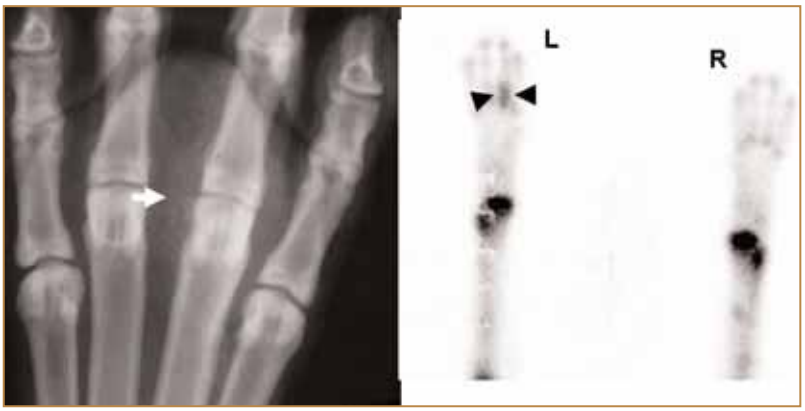

Figure 7. Arthrotic changes in the area of a metacarpal joint (arrow) producing a hot spot on the corresponding scintigraphic image (arrow heads). *Compare the left and right side.*

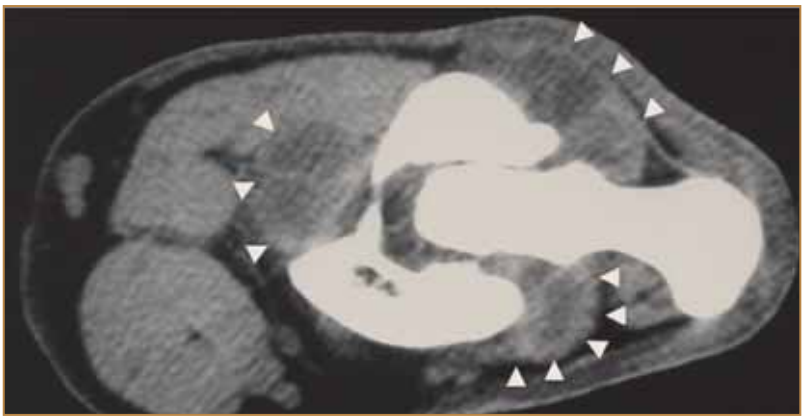

Figure 8. Soft tissue window of an elbow with severe joint effusion. *The distended joint capsule is clearly visible (arrow heads) and a distinction between joint fluid (dark areas) and synovial tissue (grey areas) can easily be made.*

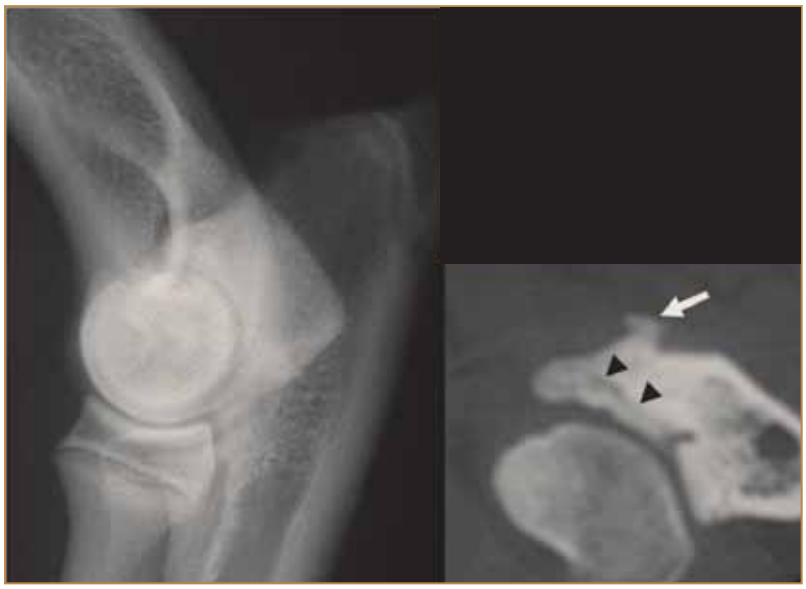

Figure 9a + b. Medio-lateral radiograph (a) and corresponding CT image (b) of a canine elbow.
On the radiograph no degenerative changes can be noticed but on the CT view a fissure line (arrow heads), subchondral sclerosis and an osteophyte (arrow) in the area of the medial coronoid process are clearly visible.

detected in an earlier stage than on conventional radiographs (FIGURE 9).

High-resolution micro-computed tomography

Until recently, three-dimensional imaging of osteoarthritis has been limited to late stages of the disease. Micro-computed tomography (micro-CT) is a new imaging tool that offers features for diagnosis of earlier disease stages and for disease monitoring. It provides spatial resolution of less than 100 micron, but the size of the objects that can be scanned is restricted to a few centimetres. The strength of micro CT is the excellent visualisation of bone. Its main applications in OA, therefore, are the analysis of human bone biopsies and of whole bone in small-animal models. With micro-CT it is possible to monitor prominent bony alterations such as osteophyte formation, trabecular remodelling, subchondral bone plate thickening, and subchondral sclerosis.

Magnetic resonance imaging

MRI has clear advantages over CT in delineating peri-articular and intra-articular soft tissue structures. Unlike other imaging modalities such as radiography, arthrography, CT and scintigraphy, MRI enables simultaneous visualisation of all components of the joint and can detect a wide variety of joint abnormalities. A major advantage is its ability to evaluate the various joint components with the surrounding structures, and not only the surface as visualised by arthroscopy or outlined with arthrography (FIGURE 10). MRI can detect several

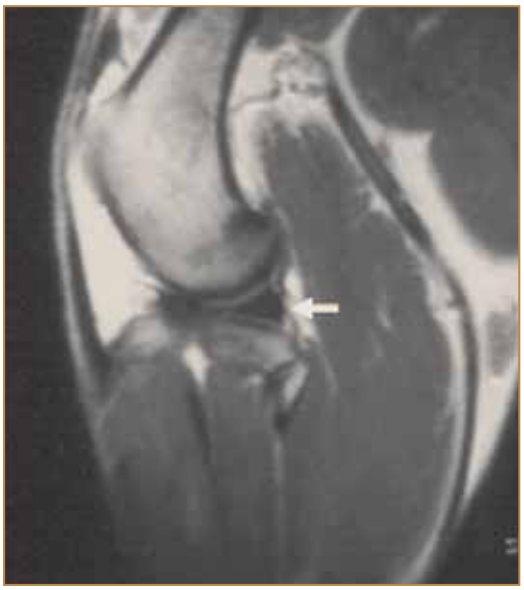

Figure 10. MRI picture of a normal canine stifle.
The soft tissues around the joint and even the meniscus (arrow) are clearly visible.

tissue characteristics and organise the signals in different combinations to produce images with a variety of appearances and allow multiplanar reconstructions. Using different sequences makes the differentiation possible between different structures and pathological processes. MRI is especially sensitive to bone marrow alterations (FIGURE 11). The current status of MRI in human medicine suggests that it would allow for evaluation of the appearance of normal and abnormal articular cartilage, but the optimal sequencing required for the detection of cartilage

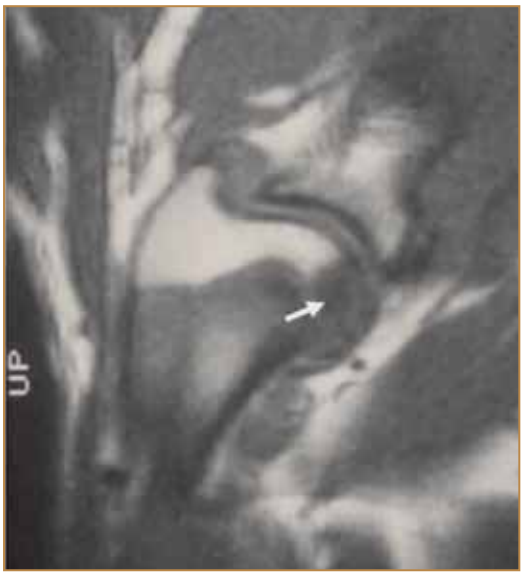

Figure 11. MRI picture of an OCD shoulder.
Notice the severe subchondral inflammatory changes within the caudal humeral head (black area). A distinction between articular cartilage and synovial fluid is not possible on this picture.

lesions still is not defined. The plethora of imaging protocols, joint-specific orientations and potential artefacts makes the design and interpretation of MRI examinations difficult. Moreover, there is a lack of consensus among radiologists as to which protocol gives the best image of articular joints. In addition, the very thin articular cartilage of dogs seems to make visualisation of the cartilage and its lesions even more difficult. The distinction between cartilage and synovial fluid is not clear, particularly in young dogs, because of insufficient contrast between the joint fluid and the articular cartilage (FIGURE 11). Although intravenous injection of Gadolinium-containing contrast agents can be useful to detect inflammatory processes, intra-articular administration of is not very helpful. Musculoskeletal MRI specialists therefore are looking forward to the specific improvements in visualisation of the articular cartilage that 3-tesla will bring to orthopaedic imaging.

Ultrasound

Ultrasound is potentially a valuable imaging technique for the musculoskeletal system in dogs and cats. Linear transducers with frequencies higher than 7.5 MHz are used because of their flat application surface and high resolution power. With this technique imaging of joints, especially of the soft tissues (e.g. ligaments and capsule) and of the articular cartilage, can be obtained. Accurate examination of joints requires substantial experience with ultrasonography and a standardised examination procedure. In most joints, the accumulation of even small amounts of fluid (hypo- to anechoic) in the region of the joint pouches can easily be demonstrated. The subchondral bone is visible as a hyperechoic line with a strong acoustic shadow. Arthritic new bone

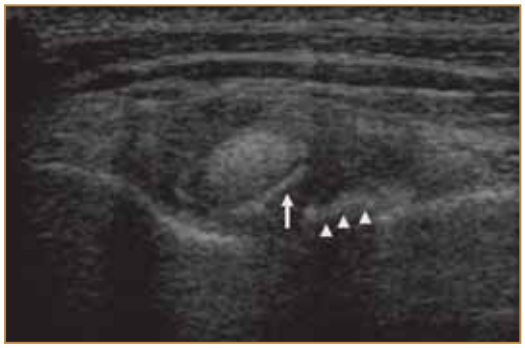

Figure 12. Transverse ultrasound of a bicipital groove of a dog with arthrosis.
An osteophyte (arrow) impinging the biceps tendon and irregularities within the groove (arrow heads) are visible.

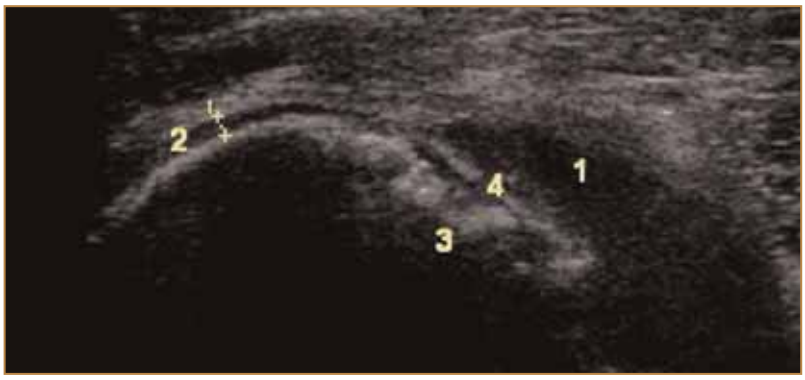

Figure 13. Ultrasound of an OCD flap in the area of the caudal humeral head of a dog.
1 = joint fluid; 2 = normal articular cartilage; 3 = the irregular subchondral crater; 4 = hyperechoic line pathognomic for the presence of a flap

formation can be picked up as irregularities on the bony surface and can be detected in an early stage (FIGURE 12). The surface of normal joint cartilage appears as an anechoic layer and is examined for its integrity. For example, cartilage defects associated with osteochondritis dissecans in the lateral femoral condyle or the humeral head show irregular borders with pronounced contractions. The presence of a second hyperechoic line at the bottom of the subchondral defect seen on ultrasound is pathognomic for the presence of a flap (FIGURE 13). Synovial proliferation can be evaluated as well (FIGURE 14).

Ultrasound biomicroscopy (UBM) is a new technology, which uses very high frequency ultrasound (20 to 55 MHz or more), compared with

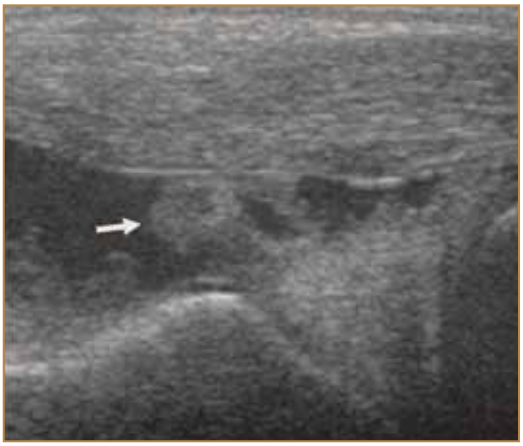

Figure 14. Synovial proliferation (arrow) in the area of the medial recess of the medial femuro-tibial joint of a horse.

the three to 15 MHz used in conventional clinical ultrasound systems. The spatial resolution of a two-dimensional image is up to about 50 microns with a penetration depth of approximately 20 mm. UBM of articular cartilage reflects the histological

structure and can accurately detect early changes such as fibrillation. UBM has the potential to becoming a valuable tool for in vivo identification of early OA lesions.

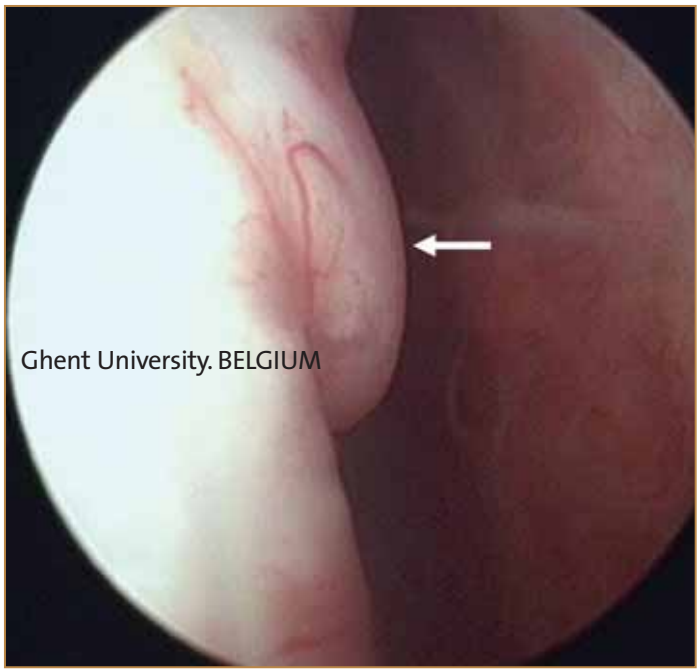

Figure 15. Arthroscopic view of an arthritic stifle of a dog.
An osteophyte (arrow) in the area of the lateral femoral condyle is clearly visible.

Arthroscopy

Arthroscopy is an excellent technique to visualise articular structures not visible on radiographs. The magnification factor of the arthroscope allows for detailed inspection of the articular cartilage, synovial membrane and intra-articular ligaments and structures (FIGURE 15).

Arthroscopy allows to taking biopsies of the different structures, can detect degenerative lesions before they show on radiographs, and can replace an exploratory arthrotomy of arthrotic joints. The drawbacks of arthroscopy are the rather steep learning curve and the relatively high cost of the equipment.

CONCLUSION

Diagnostic imaging is central to staging the severity of osteoarthritis and assessing the efficacy of therapy. An ideal imaging technique would detect changes in articular cartilage, where the primary pathology of the disease takes place. Plain radiographs are the simplest and most readily available means of joint evaluation, and the development of micro-focal radiographs, which magnify the radiograph, made joint evaluation more accurate. However, radiography, nuclear medicine scans, arthrography, and CT scans are limited in their use because they are unable to detect early cartilage abnormalities. MRI has the advantages of multiplanar imaging, better soft tissue contrast, and of being non-invasive. Like radiography, MRI may underestimate the extent of cartilage abnormality. The most sensitive technique for measuring superficial articular abnormalities is arthroscopy but it is an invasive technique. To detect early lesions and monitor their evolution in time with imaging, higher resolution is necessary. More work needs to be done in field of high resolution and volumetric MR imaging of the articular cartilage and UBM also has promising potential.

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Clinical Pharmacology of NSAIDs: Current Status and Future Prospects

HISTORICAL ASPECTS: THE NEED FOR NEW AGENTS

In the beginning was the word and the word was Willow, and extracts of the bark and the leaves were used to treat inflammatory and painful conditions at least 1500 years BC. Such use for the treatment of colic and gout was described by Dioscorides in his Pharmacopoeia in the first century AD. These were the historical antecedents of the modern range of synthetic non-steroidal anti-inflammatory drugs (NSAIDs), the first of which, sodium salicylate, was used in human medicine in 1875. Veterinary use soon followed. These moves from materia medica to synthetic drugs arose from recognition in the middle years of the 19th century that the therapeutic properties of the willow (analgesic, anti-inflammatory and antipyretic) were attributable to the glycoside saligenin, a component of which is salicyl alcohol. In 1898 aspirin was introduced as the acetyl derivative of salicylic acid by the Bayer Pharmaceutical Company and the rest, as they say, is history. Aspirin has become the most widely used drug of all time.

Until the late 1960s, the range of NSAIDs available for veterinary use was largely restricted to aspirin and phenylbutazone, together with two other drugs also of the phenylbutazone sub-group, dipyrone and isopyrin. Then in the 1970s flunixin and meclofenamate were introduced into equine medicine as potent analgesics of the fenamate sub-group of NSAIDs and flunixin subsequently became available for use in companion and farm animals. In the last 15 or so years these drugs have been supplemented by eltenac, meloxicam, nimesulide, tolfenamic acid and three drugs of the 2-arylpropionate sub-group, carprofen, ketoprofen and vedaprofen, each with a veterinary marketing authorisation. Therefore, a range of licensed drugs in both oral and parenteral formulations have been available for veterinary use for several years. Why then has there been, over the last 10 years in particular, an intensive search for novel agents within the NSAID class? There are several reasons.

First, there has been the recognition that mammals feel pain and almost certainly suffer pain in a manner similar to the human animal. The debate on animal pain has been stimulated and supported by all members of the veterinary community, including academic anaesthetists, pharmacologists, surgeons and orthopaedic specialists, as well as general practitioners and colleagues in the pharmaceutical industry. Secondly, it has been recognised that available drugs, whilst commonly very efficacious, do not consistently in all patients and in all circumstances provide adequate levels of analgesia, particularly when pain is severe. Thirdly, the need to improve safety margins has been recognised, with particular regard to gastrointestinal tolerance but also the need to achieve improved renal safety and to avoid any possibility of uncontrolled haemorrhage. In other words, high efficacy and a wide safety margin in both short and long term use have not been ensured with existing drugs in all patients or all circumstances. Whilst much less evidence is available from toxicological and pharmacovigilance studies in animals, the data on gastrointestinal toxicity of NSAIDs in humans is compelling. In the USA the **ARAMIS** (Arthritis, Rheumatism and Aging Medical Information System) study indicated that 117,000 hospitalisations per year arise from NSAID-induced gastrointestinal side-effects and there are at least 16,500 annual deaths.[1] For the United Kingdom, the estimated comparable annual human death rate is 2,000 per annum.

THE VETERINARY NSAID MARKET

The world veterinary NSAID market displays strong geographical dispositions, with 61 per cent of the total occurring in North America, 29 per cent in Europe, 3 per cent in Australia and 2 per cent in Japan. The small animal populations in North America and Europe are estimated to be 62 and 32 millions (dog) and 71 and 34 millions (cat), respectively. It is of interest further to note that 40 per cent of the EU market is in the United Kingdom (with a human population of 60 millions), whereas in France and Germany (with respective human populations of the order of 60 and 90 millions) the corresponding figures are 15 and 18 per cent. Moreover, in the United Kingdom there has been 100 per cent growth in the veterinary NSAID market since 1998, under the influence of animal welfare concerns, a high percentage of breeds disposed to osteoarthritis, a high percentage of surgical cases, an increasing level of NSAID usage in the cat and the early introduction and hence availability of new drugs e.g. carprofen and meloxicam to the UK market.

CLINICAL PHARMACOLOGY OF NSAIDS

Pharmacology may be defined as a study of drug action on the body (PHARMACODYNAMICS) together with a study of drug absorption into, distribution within and elimination from the body (PHARMACOKINETICS). Both components are crucial to the effective and safe use of NSAIDs therapeutically.[2,3] A fundamental question is, do we recognise and can we explain inter-species, inter-breed, intra-breed, inter-animal and intra-animal variation in responses to NSAIDs. For the clinician and animal owner, the answer is clearly yes and the pharmacologist offers the explanation that differences in pharmacodynamics and pharmacokinetics, together with variations in physiological/pathological state, age, etc. explain these differences.

Pharmacokinetics

Species differences in pharmacokinetics are the rule rather than the exception in that, whilst plasma protein binding is high and volume of distribution is low with almost all drugs in all species, elimination rates vary markedly, giving rise to differences in clearance and terminal half-life. The example of phenylbutazone is presented in TABLE 1 but it should

TABLE 1

	Elimination half-life	Clearance (mL/h/kg)
Man	96	-
Cow	42-65	1.24-2.90
Sheep	18	-
Goat	16	13.0
Camel	13	4.9-10.0
Horse	4-6	16.3-26.0
Dog	4-6	-
Rat	2.8-5.4	35-86
Donkey	1-2	170

Table 1. Species diferences in phenylbutazone pharmacokinetics.

be noted that marked species differences exist for most NSAIDs.

For the 2-arylpropionate sub-group of NSAIDs (carprofen, ketoprofen and vedaprofen) the pharmacokinetics are further complicated by the fact that these agents exist in two enantiomeric forms and the licensed products are the racemic (50:50) mixtures of the two. *In vivo* the disposition of the two enantiomers varies markedly. This impacts on clinical response, because only the S-enantiomer is believed to be pharmacologically active.

There is well recognised inter-animal variation in the pharmacokinetics of all NSAIDs as illustrated by data from our laboratory for S(+) carprofen in various canine breeds and for firocoxib in Beagles (TABLES 2 & 3).[4]

TABLE 2

Day of Dosing	Cmax (µg/mL)		AUC (µg/mL.h)	
	Mean	Range	Mean	Range
1	2.95	1.78-3.86	27.4	21.7-35.0
7	3.94	0.00-5.10	35.3	0.0-51.3
28	3.95	1.80-6.72	37.1	14.6-61.2

Table 2. Inter-animal variation in S(+)-carprofen pharmacokinetics in the dog†.
† Six dogs, aged 0.7 to 12 years with osteoarthritis, various breeds; 2 mg/kg BW of RS (±) carprofen for 28 days (Lipscomb et al., 2002) (RS = racemic).

TABLE 3

Parameter	Mean	Range
Cmax (µg/mL)	1.01	0.51-1.37
Tmax (h)	2.63	0.79-4.45
AUC (µg/mL.h)	11.00	8.55-14.27
V/F (L/kg)	4.21	2.78-5.08
$T^1/_2$el (h)	6.31	3.31-9.99

Table 3. Inter-animal variation in firocoxib pharmacokinetics in the beagle dog†.
† Eight young Beagles, both sexes: 5mg/kg single dose (Lees et al., unpublished data).
C = concentration; Tmax = time to reach Cmax; AUC = Area under the Curve; V/F = Volume distribution scaled by bioavailability; $t^1/_2$el = terminal half life.

As well as these inter-animal and between breed differences, a single report on celecoxib in a colony of 245 Beagle dogs revealed a within breed difference, such that the population, on the basis of capacity to eliminate the drug, could be classified into two sub-populations, an EM phenotype for which mean half-life was 1.72h and clearance was 18.2 mL/kg/min and a PM phenotype for which corresponding values were 5.18h and 7.15 mL/kg/min.[5] Clearly, this one example points to the possibility and indeed likelihood of significant genetic differences in clearance and terminal half-life between and within canine breeds. In veterinary medicine the science of pharmacogenetics is in its infancy and there is clearly a need for further studies.

Pharmacodynamics

A second basis for the variation in clinical response between species, breeds and individual animals is in NSAID pharmacodynamics. This can be considered at several levels, molecular, cellular, tissue and whole animal. ***Whole animal responses*** are the analgesic, antipyretic, anti-inflammatory and anti-thrombotic actions of NSAIDs and actions at the ***molecular level*** involve several enzymes and pathways, although the major action is recognised to be inhibition of enzymes in the arachidonic acid cascade.

Current evidence suggests that a high level of inhibition of inflammatory mediators is required to achieve clinical responses (analgesia etc.), so that 80 or even 95 per cent PGE2 inhibition may be required for effective suppression of lameness in the osteoarthritic dog.

Our understanding of the pharmacodynamics of NSAIDs was transformed in 1971 when Vane discovered that the principal mechanism of action was inhibition of cyclooxygenase (COX) leading to inhibition of synthesis of pro-inflammatory mediators of the prostanoid group such as PGE_2. Our understanding was further enhanced in 1991 with the recognition that two COX isoforms exist and these are now referred to as COX-1 and COX-2. It was immediately recognised that almost all classical NSAIDs inhibit both isoforms: COX-1 inhibition producing toxic effects, and COX-2 inhibition providing therapeutic effects. COX-1 was classified as a constitutive enzyme present in most cells of the body (erythrocytes are an exception) and responsible for physiological/housekeeping

functions, such as gastro- and reno-protection and blood clotting COX-2 was classified as an inducible enzyme, absent from or present in very low basal levels but markedly up-regulated at sites of inflammation and responsible for producing pro-inflammatory mediators.

The COX story as perceived in 1991 is still accepted by most workers but it has now been modified in several respects.

1. Recently ***a third isoform***, COX-3, was isolated from dog brain and it has been suggested that it might be involved as a central mediator of pain. It has been further suggested that, as COX-3 is a splice variant of COX-1, it might more properly be described as a COX-1 sub-type, e.g. COX-1a. Moreover, it is not clear that COX-3 exists in all species or what its actual function is. Nevertheless, the interesting proposal has been made that certain older NSAIDs e.g. paracetamol, dipyrone and isopyrin may be preferential inhibitors of COX-3 (see below for definition of terms). Furthermore, this hypothesis could account for a central analgesic action of paracetamol with little peripheral effect either as an anti-inflammatory agent or in producing deleterious effects on the gastrointestinal tract (GIT).
2. Contrary to the 1991 understanding, it is now recognised that ***COX-2 is also a constitutive enzyme***, in brain, kidney, ovary, uterus, ciliary body and bone for example. Therefore, it might be predicted that complete inhibition of COX-2, especially over long periods, might be associated with such side-effects as abortion, foetal abnormalities, delayed bone healing, delayed healing of soft tissue, cardiovascular events and reno-toxicity. In practice, the widespread use of COX-2 inhibitors in humans has been associated with a generally good safety profile for GIT and other organs. However, there remains a question mark over the 'cardiovascular events' associated with their clinical use, involving the withdrawal of one drug of this class, rofecoxib. COX-2 is constitutive in endothelial cells and its selective inhibition might disturb the endothelial PGI_2 (anti-aggregatory and vasodilator via COX-2) platelet TxA2 (pro-aggregatory and vasoconstrictor via COX-1) balance in the direction of platelet aggregation and vasoconstriction. The 'cardiovascular events' in humans, which have been the subject of much recent public discussion, might reflect this imbalance.
3. It has been suggested that ***COX-1 contributes to*** the synthesis of ***pro-inflammatory prostaglandins***. In knock out mice with the COX-1 gene deleted there was a reduced inflammatory response. Therefore, both COX-1 and COX-2 inhibition, as provided by the older non-selective NSAIDs, might be required for optimal efficacy. However, this is

TABLE 4

Classification	Example	Comment
Preferential or selective COX-1 inhibitors	ketoprofen† vedaprofen†	
Non-selective COX inhibitors	phenylbutazone flunixin tolfenamic acid ketoprofen† vedaprofen†	• No significant biological or clinical differences in concentrations producing COX-1 and COX-2 inhibition
Preferential and selective COX-2 inhibitors††	carprofen celecoxib deracoxib etodolac meloxicam nimesulide	• COX-2 inhibition potency is 5 to 100-fold greater than COX-1 inhibition • Some anti-inflammatory and analgesic activity at concentrations that inhibit COX-2 but not COX-1 • At higher concentrations, clinically significant COX-1 related adverse effects may occur
Specific COX-2 inhibitors†††	etoricoxib firocoxib lumiracoxib	• Greater than 100-fold preference for COX-2 inhibition • No inhibition of COX-1 *in vivo* (no G.I. ulceration or platelet effect) even at maximal therapeutic dose
Dual COX and 5-LO inhibitors	tepoxalin	• Blockade of COX and 5-LO with concentrations achieved with clinical dose rates

Table 4. COX inhibitor classification.

† *Differing data from studies in various laboratories*

†† *Possible species differences*

††† *Selectivity versus specificity depends on (a) position and (b) slope of COX-1 and COX-2 inhibition curves and (c) on the level of inhibition considered e.g. IC50, IC80, IC95, etc.*

controversial and most experimental and clinical data suggest that selective COX-2 inhibitors are as efficacious as non-selective NSAIDS.

4. With the introduction of a ***novel class of NSAIDs, the dual inhibitors*** which inhibit two enzymes in the arachidonic acid pathway, COX and 5-lipoxygenase (5-LO), it has become necessary to compare the older NSAIDs with this new drug class.

Interpreting published data in this field presents problems as the literature has reported major differences in potency ratios (COX-1:COX-2) for individual drugs (TABLE 4). It should be noted that the *in vitro* conditions used to determine COX activity impact on the COX-1:COX-2 inhibition ratios, with much higher values (i.e. higher selectivity for COX-2) commonly obtained when isolated enzyme, broken cell or intact cell in buffer determinations are used in comparison with whole blood assays. The latter are regarded as the gold standard and most relevant to conditions in the whole animal.

A further complication is that there may be species differences in drug potency for COX-1 and COX-2 inhibition and therefore COX-1:COX-2 inhibition ratios. For example, on the basis of published data from whole blood assays carprofen might be classified as COX-1 selective or preferential in man, non-selective in the horse and COX-2 preferential or selective in the dog and cat (TABLE 5). Data interpretation must also take account of possible differences in the slopes of COX-1 and COX-2 inhibition curves as well as the relative positions of the curves. In feline whole blood studies conducted in our laboratory, the IC_{50} (Inhibitory Concentration 50) inhibition ratio values were 3.1 and 25.6 for meloxicam and S-carprofen, respectively, whilst for IC_{80} the corresponding inhibition ratios were 21.4 and 64.9.[11] The implications of these data for efficacy and safety in clinical use have not been determined but, on the basis of *in vitro* inhibition ratios and drug concentrations in plasma *in vivo*, it is likely that carprofen inhibition of COX-2 is weak or moderate in the dog. This had led to the suggestion that carprofen may act, in part, by one or more non-COX mechanisms. On the other hand, COX-2 inhibition is predicted to be complete and long lasting in the cat at the manufacturer's recommended dose rate, and optimal efficacy might be achieved with a lower dose than that recommended.

TABLE 5

Species	Enantiomer	IC_{50}	IC_{80}
Human[1]	RS	0.02	0.253
Canine[2]	RS	16.80	101.20
Canine[3]	RS	7.00	6.00
Canine[4]	RS	5.40	-
Canine[5]	S	25.00	-
Canine[5]	R	2.40	-
Equine[5]	S	1.70	-
Equine[5]	R	2.70	-
Feline[6]	S	25.60	64.90

Table 5. Whole blood assays for RS-, S- and R-carprofen COX-1:COX-2 IC_{50} and IC_{80} ratios.

[1] Warner et al. (1999); [2] Streppa et al. (2002); [3] McCann et al. (2004); [4] Wilson et al. (2004); [5] Lees et al. (2000); [6] Giraudel et al. (2005). [R = right; S = sinister (left); RS = racemic].

CONCLUSION

In summary, the available data on NSAIDs indicates distinct species, within breed, between breed and inter-animal differences in pharmacokinetics, notably clearance and terminal half-life. Although pharmacodynamic data are more limited, it also seems likely that similar species, breed, and between animal differences may occur in parameters such as IC_{50}, IC_{80}, etc. for inhibition of COX-1 and COX-2. These two aspects of each drug's pharmacological profile will together account for the between animal differences commonly encountered by clinicians and owners in therapeutic responses and tolerance to NSAIDs. A drug which clinically best suits one animal will not suit the next, and an agent with poor efficacy or low tolerance in a particular animal will be efficacious and well tolerated in another animal. Clinical assessment and judgement are still required with available agents.

NOVEL NSAID CLASSES

Drugs of the NSAID class which may be described as novel are listed in TABLE 6.

Drugs with **preferential**, ***selective or specific activity against COX-2*** have provided a major advance in pain therapy.[9] Based on whole blood canine assays carprofen, deracoxib, meloxicam and nimesulide may be classified as preferential or selective NSAIDs, whilst firocoxib is a specific inhibitor in this species. COX-2 specific inhibitors are

TABLE 6

Drug Class	Characterisitics
Selective and specific COX-2 inhibitors	• Inhibition of COX-2 with absence or only limited inhibition of COX-1 at recommended dose rates. • Improved GIT safety profiles (see TABLE 4).
Cyclooxygenase inhibiting nitric oxide donors (CINODs)	• Nitrosoesters of NSAIDs (e.g. aspirin, indomethacin, phenylbutazone), releasing nitric oxide in vivo. • Improved GIT safety profile and possibly enhanced efficacy.
Dual COX, 5-LO inhibitors	• Inhibition of both COX and 5-LO thus blocking at therapeutic dose rates the synthesis of two groups of inflammatory mediators derived from arachidonic acid: prostaglandins and leukotrienes. • Improved GIT and possibly renal safety profiles.

Table 6. NSAIDS with novel pharmacodynamic properties.

of particular interest in view of the introduction of several COX inhibitors into human medicine. The availability of firocoxib in canine medicine for the therapy of canine osteoarthritis constitutes the first COX-2 specific drug for veterinary use. [8] The very high COX-1:COX-2 potency ratio of 380:1 for this drug ensures that there will be no COX-1 inhibition at therapeutic dose rates. The improved GIT tolerance of selective COX-2 inhibitors over non-selective inhibitors is not universally agreed, but is nevertheless well established. [6] However, when GIT ulceration has occurred from any cause, COX-2 is rapidly induced and generates cytoprotective vasodilator prostaglandins. Moreover, COX-2 inhibitors have been shown experimentally to inhibit the healing of ulcers in rodent studies. It might, therefore, be suggested that selective and specific COX-2 inhibitors are unlikely to cause ulcers but they could delay the healing of pre-existing ulcers. Further evaluation of this possibility is required.

Cyclooxygenase inhibiting nitric oxide donors (CINODs) are another class of novel agents. They will not be further considered, as there is no immediate likelihood of introducing a drug of this class into veterinary therapeutics.

A third area of advance is ***dual inhibition***. Dual inhibitors are those agents which inhibit both COX and 5-lipoxygenase (5-LO). The licensed drug, tepoxalin, has now been used in canine medicine for approximately 2 years and licofelone is a dual inhibitor developed for human use. [12-17]

Tepoxalin is converted in vivo to an acid metabolite, which is a potent COX inhibitor and which is cleared more slowly than the parent compound. It produces most of its COX inhibitory effect via the metabolite. However, the parent compound also produces 5-LO inhibition. This gives tepoxalin a potentially broader spectrum of anti-inflammatory activity than that of COX inhibitors.

As well as inhibiting COX and 5-LO, tepoxalin inhibits another lipoxygenase enzyme, 12-LO, and blocks the release of IL-2, IL-6 and TNFα. Whether these actions occur with clinical dose rates is not known but potentially they provide additional bases for a broad anti-inflammatory spectrum. Clinical trials comparing tepoxalin with carprofen and meloxicam in canine osteoarthritis have revealed similar efficacy at clinically recommended dose rates for signs reflecting analgesia and relief of lameness. This may be due to a relative insensitivity of the pain indices used to discriminate between drugs or it may be that mediators generated by COX rather than 5-LO cause the pain associated with osteoarthritis. Therefore, the benefit provided by tepoxalin is mainly the greater GIT (and possibly renal) tolerance. The GIT tolerance of dual inhibitors differs from that of the older non-selective COX

inhibitors. In pre-clinical toxicity studies, beagle dogs receiving up to five or 10 times the therapeutic dose over periods of many months have not displayed major GIT side-effects. The explanation for this probably lies in the dual inhibition phenomenon. When NSAIDs inhibit COX there may be shunting of the substrate, arachidonic acid, towards 5-LO. This may increase the synthesis of leukotrienes with vasoconstrictor properties and which cause neutrophils to adhere to gastric mucosal blood vessels. The potential consequence is mucosal ischaemia and the release of tissue damaging mediators from neutrophils. Thus, leukotrienes may contribute to the GIT side-effects of NSAIDs. By blocking the leukotriene synthetic pathway, tepoxalin may remove this mechanism of GIT based toxicity.

The pharmacokinetics of tepoxalin are of interest not only because of the synthesis of an active metabolite but also because the absorption of parent drug from the GIT is affected by food. Its bioavailability is significantly increased when administered with a high fat meal in comparison with dosing with a low fat meal or on an empty stomach. [18] Therefore, to achieve optimal efficacy with this drug, attention should be paid to dosing in relation to feeding schedules, with the drug preferably administered with feed.

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Putting Theory into Practice - Best Practice Management for Osteoarthritis

INTRODUCTION

Osteoarthritis (OA) is an insidiously progressive disease producing pain and loss of function in affected joints. The suffering and restrictions on exercise that the active disease produces, make a profound impact on the quality of life of the patient. Fortunately, although the disease is widespread, estimated to affect one in five of all dogs, it is not always associated with debilitating disease.[1] Indeed it has been speculated that only animals with moderate to severe OA pathology are identified and presented as clinical problems.[2] These facts set the scene for any discussions aimed at identifying management strategies for the disease.

OBJECTIVES OF OSTEOARTHRITIS MANAGEMENT

The goals for management of OA can be identified in global terms as [3,4]

1. Controlling Pain
2. Maintaining and improving the range of movement and stability of affected joints
3. Limiting functional impairment of the patient

These general objectives should underpin any attempts at management. OA is a complex disease and a very clear understanding of the disease process is essential to advise selection of treatment. It has been suggested that best results in human patients are obtained by an individualised and patient-centred approach involving multiple strategies.[5] It would appear that management of the condition in animals is no different. This type of approach delivers the necessary focus to sustain successful management of any affected patient through meeting long-term requirements.

RECOGNISING OSTEOARTHRITIS AS A PROBLEM

The simplest method of recognition is by radiographic review; however there is poor correlation between radiographic appearance and the extent or even existence of a significant clinical problem in an OA joint.[6] Radiography is vital to

confirm the existence of disease in a joint and to eliminate other possible causes of clinical signs (FIGURE 1). It should not be used to estimate the clinical severity of the condition.

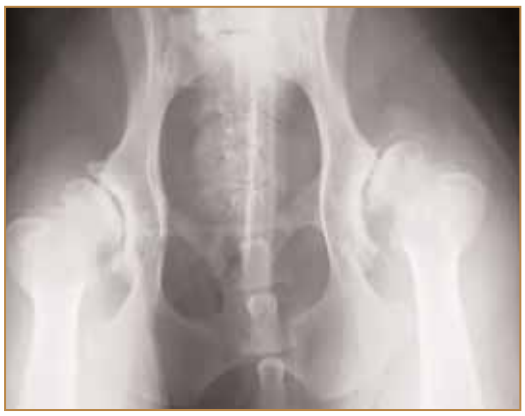

Figure 1. Hip X-Ray.
Radiographic features of OA can be distinctive but are not closely related to function.

Many of the signs accompanying OA are caused by pain and evaluation of this is best achieved by observation and clinical assessment. The classical signs are listed in TABLE 1. It is the appearance and identification of these signs and how obvious they are that guides the clinician to judge the severity of the disease. These are also used to judge the effectiveness of any management strategy employed. Increasingly, alterations of behaviour indicative of chronic pain are also being assessed as more subtle indicators of an ongoing problem. [7] Assessment of patient behaviour is difficult in the consulting room and relies on the observational skills of the owner (FIGURE 2). In studies attempting to evaluate the accuracy of these owner observations, they were found to compare very favourably with objective assessment of disability provided by force plate measurements on the same animals. [8,9] This information is extremely useful to allow construction of plans to assist measures to alleviate the consequences of the disease.

TABLE 1

Lameness	Crepitus
Stiffness	Swollen joints
Reduced movement in joint	Muscle atrophy
Reluctance or difficulty with execise	

Table 1. Classical signs of osteoarthritis.

OSTEOARTHRITIS AS A CHRONIC DISEASE

It is worth considering the consequences of the chronic nature of OA and how this impacts on the disease itself and any attempts at treatment. OA remains a slowly progressive condition fuelled by constant release of inflammatory mediators from a chronic low grade inflammatory reaction. Constant stimulation of nociceptive receptors in affected joints can lead to an altered perception of pain with hypersensitivity, hyperalgesia, allodynia and genetic alteration in central transmission pathways to enhance pain sensation. [10,11] This means that small or even normal stimuli such as normal joint movement can be perceived as painful as a result of physiological re-programming. This can be a very difficult state to reverse once well established.

Figure 2. Great Dane with OA.
Aged behaviour can be a result of chronic pain due to an underlying osteoarthritis problem.

The other, more obvious, consequence of chronic joint debility and inflammation is the increasing involvement of the structures surrounding the joint, producing a much more complex pathological equation. Muscles will atrophy very rapidly as a result of lack of activity and reflex neurogenic feedback stimulated by intra-articular pain. Muscles can suffer focal damage with mediator release and so become a source of diffuse, poorly localised pain. Muscle wastage can also increase problems and pain in the joint by reducing protective support. Changes in subchondral bone can also result in increased pain. [12] Fibrous thickening of joint capsule, ligaments and tendons can accompany muscle wastage. Fibrosis and changes in joint shape caused by new bone formation will produce stiffness and alter joint movement.

All of these events add to the cyclic deterioration of the osteoarthritic joint but also create an urgent need for early intervention to avoid escalation of the pathology and so maintain reversibility of clinical signs. The more advanced the condition the more difficult it is to treat.

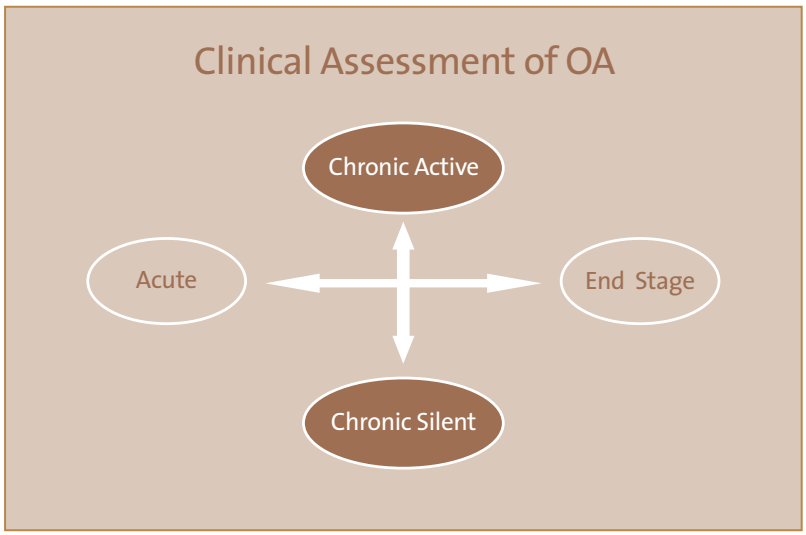

Figure 3. Phases of osteoarthritis.

AIMS OF MANAGEMENT

OA is a dynamic condition in which a number of different clinical phases can be recognised (FIGURE 3). Three clearly recognisable phases are '*Chronic Phase Silent Disease*', '*Chronic Phase Active Disease*' and '*End Stage Disease*'. The phase will be determined by the pathological changes already present within the joint and the pain that the animal is experiencing. Several examinations conducted at different time intervals are necessary to properly establish this judging the success or failure of different control measures.

Looking at this model (FIGURE 3) there is a gradual deterioration of the affected joint to end stage disease. The clinical picture may be punctuated by periods of '*active*' disease, where clinical signs are apparent, and '*silent*' disease, where there are few or no clinical signs and no pain. Using this judgement the initial aim of management will be to try to convert active joints to clinically silent phase where signs are minimal. The second aim will be to keep them in this state. The third aim will be to slow the progression along the horizontal axis to end stage disease.

OPTIONS FOR THE MANAGEMENT OF OSTEOARTHRITIS

There are a number of methods that have been used in the past to good effect and continue to be the main components of any strategy designed to reduce the effects of osteoarthritis in a patient. These can be placed in broad groups as follows

1. Non-pharmacological methods
2. Pharmacological or Medical methods
3. Surgical Interventions

The majority of animals with clinical OA are managed without surgery, but surgery can be the best option depending on the joint involved (hips) and state of pathology. Euthanasia is an option which must be considered in intractable cases.

Non-pharmacological methods

Non-pharmacological methods can be divided into 1) dietary restriction or manipulation, often to achieve weight loss, 2) instructions about mobility including warm-up exercises, exercise plans, physiotherapy and hydrotherapy, and 3) a third group concerned with mechanical aids to assist or facilitate movement and common sense measures to minimise discomfort. The application of these methods, with the possible exception of dietary intervention to lose weight (FIGURE 4), is often haphazard and poorly maintained in the animal population.

Pharmacological methods

The main group of medical agents used to gain control of the signs of OA are the Non-steroidal anti-inflammatory agents (NSAIDs). These inhibit the cyclo-oxgenase enzyme, which is a key component in the arachidonic acid cycle of inflammation. In the management of human OA, NSAIDs are widely used and are the most frequently requested by arthritis sufferers. We are very fortunate to have a range of reliable and relatively safe NSAIDs licensed for use in dogs. As such, many strategies for OA management centre on these agents. As toxicity is a significant risk with NSAIDs strategies have to be constructed around avoiding toxic side-effects.

Other medical agents used include corticosteroids, again for anti-inflammatory effects, and opioids for pain relief.

There has been an increasing trend to identifying and employing agents which may modify the articular cartilage, synovial fluid and synovium of affected joints. These are often classed as '*Slow Acting Drugs*' for

OA or '*Disease Modifying Agents*'. Included in this group are parenterally administered polysulphated glycosaminoglycans (PSGAG), pentosan polysulphate and hyaluronic acid. The biggest group of agents in the group are the orally administered so-called '*nutraceuticals*', including glucosamine and chondroitin sulphate. There are numerous commercially available products containing these agents alone or in combination. These are often used in combination with NSAID medication.

The main problem in arthritis management is processing all of the choices available and selecting an appropriate agent to meet the objectives. Many attempts at management are based around a single drug strategy. This contradicts the evidence of the effectiveness of a multimodal approach suggested previously.

MANAGEMENT PLANS FOR OSTEOARTHRITIS

The whole process of managing osteoarthritis can be summarised as follows

1. Identification of a problem
2. Assessment of the problem
3. Review possibilities and select an intervention strategy
4. Assess success of this within a set time frame
5. Continue, modify, replace or add to intervention(s)
6. Re-assess etc. (maintenance phase)

The process must be simple to use, successful in achieving rapid success, sustainable long term and must bring the clinical problem under the control of all concerned. It must also be practical to use and economically feasible.

One way of ensuring that there is a controlled approach to the problem is by using pre-determined management plans, which are customised for each patient. These have the multimodal approach imbedded but require judgements to be made about priorities for treatment. They often combine pharmacological and non-pharmacological methods and, if properly designed, will evolve to meet the changing needs of a chronic disease process. This last point provides sustainability.

Successful plans depend on good quality assessments being made at different times during the management process. These must be repeatable and allow comparison, not only with the last assessment, but with all assessments recorded. This is the key to exerting control over chronic evolving disease processes. Records must be reliable and assessment easy to do, but also able to detect variations in the clinical state. Assessment of a complex disease like OA is not an easy feat and is by necessity largely subjective. Measuring pain and quality of life is much more difficult than evaluating range of movement and force plate measured weight bearing. Many attempts have been made to construct a scale that can be used to give repeatable measurements of pain with limited success. Carefully constructed client questionnaires seem to be the most useful way of judging the subtle changes that can indicate early improvement or deterioration.

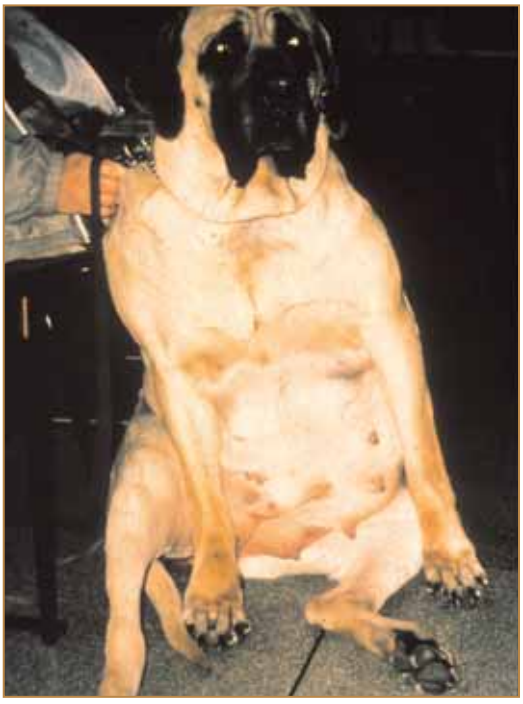

Figure 4. Bull mastiff with OA.
Weight loss in obese animals is a key requisite in any management plan.

Maintenance and management plans

A critical way in which the approach to the OA patient can be improved is to develop a maintenance approach and include it in the plan. Regular visits at set times should be arranged for the animal to be checked rather than the animal only being presented when a problem occurs. These are initiated once the presenting signs are brought under control and the plan evolves to concentrate on the long term management issues. This approach is particularly useful in chronic diseases

TABLE 2

	Primary	Secondary	Tertiary
A **Analgesia**	NSAID	Opiates Acupuncture	Other medical strategies, anti-depressants, relaxants
B **Bodyweight**	Dietary Control	Specific diets Hydrotherapy	
C **Complications** **Comfort**	Screen blood Special bed	Further medication High surveillance Mobility aids	Touch therapy, heat, massage
D **Disease**	Joint mobility Nutraceuticals	Intra-articular therapy	Modification or salvage surgery
E **Exercise**	Directed exercise	Hydrotherapy	Physical therapy

Table 2. Management plan options.
Applying these options allows complex plans to be constructed which may be necessary in the management of ongoing complex cases.

TABLE 3

	First Visit	Second Visit	Third Visit
A **Analgesia**	NSAID started at maintenance dose	NSAID dose reduced	Every other day NSAID
B **Bodyweight**	BCS 5 Targets set Diet provided	BCS 4 Diet continued Hydrotherapy	BCS 3 Maintenance diet
C **Complications**	Bloods normal Urine normal		Urine
Comfort	Special bed	Touch therapy instituted	Ramp for car
D **Disease**	Nutraceuticals started	Continue	Radiograph joint Nutraceuticals
E **Exercise**	Exercise chart position 5 Warm up exercises	Exercise chart position 4	Exercise chart position 2

TABLE 3. Maintenance plan and records.
This scheme allows complex plans to be constructed which may be necessary in the management of ongoing complex cases. BCS = body condition score using a 5 point scale.

where regular assessments can be used to map gradual progress. It also allows early identification of developing problems and rapid adjustment of the plan. The approach is particularly useful in ensuring that non-pharmacological measures are being maintained and optimised. In many cases veterinary nurses can manage a large component of the maintenance phase.

PROPOSED MANAGEMENT STRATEGY FOR OSTEOARTHRITIS

A '*Five-Point Plan*' for OA management is proposed to satisfy the requirements outlined above. The plan identifies five separate areas of management which can be addressed simultaneously to deliver a multimodal approach. These areas are ***analgesia; bodyweight, complications and care, disease*** and ***exercise*** (TABLE 2). One area should be identified as a priority at the stage of the disease. The plan simplifies the process by providing prepared options in each area and tracking these over time (TABLE 3). It is supported by feed charts, body condition score information and exercise charts as part of this preparation. Exercise charts, with a number of different levels of exercise clearly explained, are a great time saver and aid to compliance with these plans. The key features can be listed hereafter:

- It allows a multimodal plan to be set-up and implemented very easily
- All of the practice members are working from the same strategy
- Different members of the team may have different roles to play
- The owner can be informed and instructed easily
- Evolution of care progresses with the changing disease requirements
- Complex problems can be managed by extending into the secondary or tertiary options identified for each problem whilst still following the strategy. These may be non-responsive cases or cases with intercurrent disease (hepatic, renal etc.)
- It allows incorporation of new developments without altering the basic planning process.

Assessment sheets will be analysed to give a specific and global view of the progress over time. In this way a highly focused and sophisticated plan can be used and maintained with the minimum of effort but to the maximum benefit of the affected animal and owner.

Best practice can be easily delivered within realistic financial targets.

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Fatty Acids and Evidence-Based Dietary Management of Canine Osteoarthritis

INTRODUCTION

Osteoarthritis (OA) is the most common form of arthritis recognised in man and all domestic animal species. It is typically a slowly progressive condition, and it is characterised by two main pathologic processes: degeneration of articular cartilage, with a loss of both proteoglycan and collagen; and proliferation of new bone. In addition, there is a variable, low-grade inflammatory response within the synovial membrane. Current estimates of the prevalence of arthritis in senior and geriatric dogs range from 20 to 25 percent. The objective of this paper is to discuss the nutritional management of dogs with OA including the importance of weight control and the use of foods enhanced with omega-3 fatty acids.

MANAGEMENT OF CANINE OSTEOARTHRITIS

The objectives of treatment for OA are multifaceted; reduce pain and discomfort, decrease clinical signs, slow the progression of the disease, promote the repair of damaged tissue, and improve the quality of life. Current treatment modalities used to manage dogs with chronic pain due to OA include anti-inflammatory and analgesic medications, disease-modifying osteoarthritis agents (DMOAs), nutraceuticals, weight reduction, low-impact exercise programs, physical therapy, and therapeutic foods.

Medications

At present, the use of five non-steroidal anti-inflammatory drugs (NSAIDs) is firmly established in the treatment of OA in dogs, i.e. carprofen, etodolac, deracoxib, meloxicam, and tepoxalin. Treatment of OA with these NSAIDs is generally analgesic and provides rapid relief of symptoms related to inflammation, but none them is effective at treating the underlying pathology. These drugs are effective, but have proven incapable of affecting the progression of the disease. In addition, NSAIDs as a class of drugs can cause multiple side effects related to the gastrointestinal, hepatic, renal, and hematopoietic systems.

When NSAIDs are prescribed for treatment of OA, owners should be made aware of the clinical signs that may indicate the adverse effects of these products.

Disease-modifying osteoarthritis agents

DMOAs are substances considered to modify the course of OA by improving the health of articular cartilage or synovial fluid. Glucosamine and chondroitin sulfate are DMOAs and are used, either alone or in combination, for the treatment of OA symptoms. ***Glucosamine*** is naturally present in the body and is one of the basic carbohydrate components used in the synthesis of the disaccharide units that compose all of the glycosaminoglycans (GAG) found on proteoglycans in cartilage. ***Chondroitin sulphate*** is a constituent of the GAG (aggrecan) of articular cartilage and has repeating sub-units of glucuronic acid and N-acetyl galactosamine sulphate. These compounds have been used to treat symptoms of OA and provide building blocks to synthesize articular cartilage. Recent meta-analyses of glucosamine and CS for treatment of human OA yielded differing conclusions and indicated the need for further studies;[1-3] To summarise these reviews, glucosamine ingestion demonstrated efficacy in some symptom-relieving parameters and narrowing of joint space in humans. Chondroitin sulphate ingestion showed similar symptom-relieving effects, but its ability to modify the structure of articular cartilage was not confirmed. Both of these substances are used widely in companion animal foods. However, no published research is available to demonstrate a direct therapeutic effect in osteoarthritis at the levels they are currently included in most pet foods.

Nutraceuticals

There continues to be great interest in the discovery of '*natural*' products to alleviate symptoms of OA. A recent review of some of these compounds (e.g. green tea extract, Asian herbal remedies) concluded that there is a need to conduct studies and clinical trials to support *in vitro* evidence that these nutraceuticals may be beneficial to arthritis sufferers.[4] To date, no studies have been published with these compounds in companion animal models. Trials in humans and canines with OA have been conducted on the use of curcuminoids. Curcuminoids, extracted from turmeric, have some interesting anti-inflammatory effects in certain animal models and *in vitro* assays. However, a recent trial in dogs with OA failed to find a treatment effect using force plate analysis as the primary outcome variable, although veterinary surgeon assessment of response to treatment was positive.[5]

Weight reduction

It is accepted that obese dogs are at increased risk for OA. Nevertheless, the role of obesity in the aetiology of OA is not clear. Studies in humans suggest that obesity, as an isolated factor, may contribute to osteoarthritis in the knee. Increased mechanical forces across the joint, leading to cartilage matrix damage, likely explain much of this increase. Study of body weight and hip dysplasia in large breed dogs has confirmed a beneficial effect of decreased body weight in joints predisposed to OA change.[6-7] Weight reduction can help to decrease abnormal forces placed on joints and can help alleviate symptoms in the affected patient. Overweight and obese dogs that have OA should reduce their weight to a normal body condition. In addition, exercise is a vital component of weight loss for OA treatment. Frequent, mild, weight-bearing exercise over an extended period has been shown to help patients reduce body weight, increase joint mobility, reduce joint pain, and strengthen supporting muscles.

Dietetic foods

The nutrient profile of a dietetic food should meet the distinctive nutritional requirements established by medical evaluation, as being effective to manage a specific disease or condition. Dietetic foods designed for companion animals with OA need to supply age appropriate nutrition and specific nutrients that help reduce inflammation and pain, provide the building blocks for cartilage repair, slow the degradative process, compliment the prescribed medications, and provide tangible improvement in clinical signs of OA. Recent discoveries in fatty acid nutrition have provided clear evidence that canine OA may be very responsive to dietary addition of specific fatty acids.

All mammals synthesize fatty acids de novo up to palmitic acid, which may be elongated to stearic acid and converted into oleic acid (18:1n-9). Plants, unlike mammals, can insert additional double bonds into oleic acid between the omega end and the first double bond to produce the polyunsaturated fatty acids (PUFA) linoleic acid (LA;

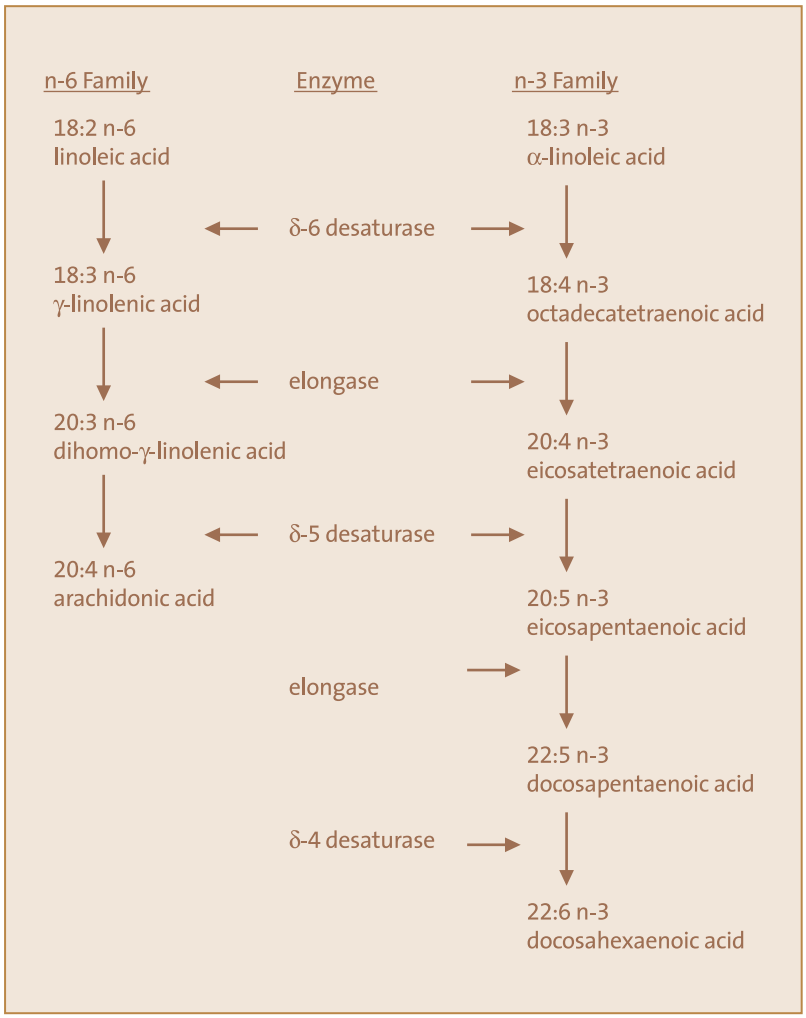

Figure 1. Metabolic transformations of two major unsaturated fatty acid families by desaturation and elongation.

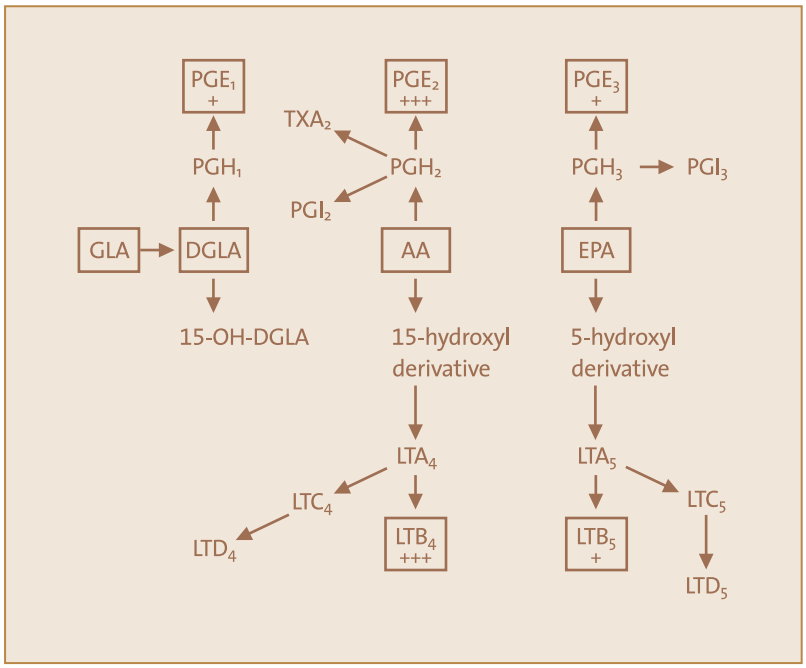

Figure 2. Potential effects of n-3 and n-6 PUFAs on inflammation.
+++ strong proinflammatory; + weak proinflammatory.

18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3). Both LA and ALA are considered essential fatty acids because animals cannot synthesize them from other series of fatty acids and must be supplied by the diet. LA can be converted into arachidonic acid (AA; 20:4n-6) via desaturation and elongation in the animal (FIGURE 1). Many marine plants, especially algae in phytoplankton, carry out chain elongation and desaturation of ALA to yield n-3 PUFAs with twenty and twenty-two carbon atoms and five or six double bonds. It is the formation of these long chain n-3 PUFAs by marine algae and their transfer through the food into fish, that accounts for the abundance of eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids in certain marine fish oils.

AA and EPA act as precursors for the synthesis of eicosanoids, a significant group of immuno-regulatory molecules that function as local hormones and mediators of inflammation (FIGURE 2). The amount and type of eicosanoid synthesized are determined by the availability of the PUFA precursor and the degree of activity of the enzyme system to synthesize them. In most conditions the principal precursor for these compounds is AA, although EPA competes with AA for the same enzyme systems. The eicosanoids produced from AA appear to be more pro-inflammatory than those formed from EPA and, when produced in excessive amounts, may lead to pathological conditions. Ingestion of oils containing n-3 PUFA results in a decrease in membrane AA levels, since n-3 PUFA replace AA in the substrate pool. This leads to an accompanying decrease in the synthesis of eicosanoids from AA, and an increase in eicosanoids derived from EPA, which promote minimal to no inflammatory activity. Inflammatory eicosanoids produced from AA are depressed when dogs consume foods with high levels of n-3 fatty acids.[8]

STUDIES

"*In Vitro*" studies

Mechanisms of cartilage metabolism in canine OA and the potential role of n-3 fatty acids to ameliorate the early events in the disease process recently were investigated using *in vitro* models.[9] These studies identified some similarities and distinct differences between cartilage from dogs

and other species in the response to catabolic agents and n-3 PUFAs. Canine cartilage proteoglycan synthesis was significantly decreased by numerous catabolic agents, however, proteolysis and loss of aggrecan, could only be stimulated by oncostatin M (OSM), leukaemia inhibitory factor, and retinoic acid. Stimulated aggrecan loss was associated with increased cleavage by aggrecanases and not matrix metalloproteinases. EPA was the only n-3 fatty acid able to significantly decrease the OSM-stimulated loss of aggrecan in the canine cartilage in vitro model.

Clinical Studies

Four clinical studies (randomized, double-masked, controlled studies) were completed recently in arthritic dogs fed either a control (Purina® Dog Chow®) or therapeutic food (Prescription Diet* Canine j/d*) designed to manage canine OA. Three studies were conducted as six month (one study) or three month prospective studies (two studies) in veterinary hospitals in the United States. A fourth study was conducted as a three month prospective study in two academic specialty practices in the United States.

In all studies, OA was diagnosed based on compatible history, clinical signs and radiographic evidence of arthritis in one or more joints on the clinically affected limb. To be eligible for inclusion, dogs also had to be at least one year of age, weigh 12.5 kg or more, consume dry dog food and be free of systemic disease as determined by history, physical examination, complete blood count, serum biochemistry analysis and urinalysis. Exclusion criteria included acute traumatic injuries, complicating disease conditions, pre-existing conditions for which corrective surgery was anticipated during the feeding period and recent intra-articular injection or arthrocentesis.

Change in arthritic condition over time was measured in these studies and was based on owner observations of clinical signs and veterinary clinical evaluations. Variables were assessed at the beginning of the study and at set time intervals after onset of feeding the control or therapeutic food. Additionally, veterinary clinical evaluations were conducted at each time interval. These consisted of an orthopaedic examination with a specific emphasis on lameness and pain, limitation in weight-bearing ability, range of motion of the affected joint(s) and willingness to bear weight on the most affected limb when the contra lateral limb was elevated. Pet owners were given the option of feeding wet and dry foods with similar levels of omega-3 fatty acids (TABLES 1 and 2).

Investigators in the three studies conducted in veterinary hospitals reported that the animals being fed the EPA supplemented therapeutic formula improved in several parameters which were evaluated during scheduled physical examinations throughout the studies. Veterinarians reported a significant improvement in range of motion and ability to bear weight, along with a decrease in pain (upon palpation of the affected joint) and lameness as compared to the arthritic condition of these dogs prior to participating in the studies. In addition, pet owners observed improvements in multiple symptoms associated with OA; rising from rest, running, walking and playing.

In the academic specialty practice clinical study, variables were assessed at the beginning of the

TABLE 1

Nutrient % dry matter	Control	Test
Protein	23.2	20.0
Fat	13.9	13.6
NFE	54.7	53.3
Total Omega-3	0.09	3.48
EPA	<0.01	0.38
Omega-6: Omega-3 ratio	22.8	0.7

Table 1. Nutrient composition of test foods; dry products.

TABLE 2

Nutrient % dry matter	Control	Test
Protein	45.8	20.8
Fat	24.4	15.0
NFE	18.8	47.8
Total Omega-3	0.16	3.45
EPA	<0.01	0.48
Omega-6: Omega-3 ratio	27.5	0.7

Table 2. Nutrient composition of test foods; wet products.

study and at 45 and 90 days after onset of feeding the control or test food. Additionally, gait analyses using a computerized biomechanical force plate were also conducted at the same time intervals. For each dog, five valid force plate trials were obtained during each test period for the most severely affected and ipsi-lateral limb. Orthogonal ground reaction forces of peak vertical force, vertical impulse, braking and propulsion peak force, and braking and propulsive force were measured and recorded. All forces were normalized with respect to body weight in kilograms and data from valid trials for each limb were averaged to obtain a mean value at each time period.

On clinical orthopaedic examination, a significantly greater percent of dogs consuming the test food were evaluated as improved versus those consuming the control food. In addition, more dogs in the test group had a reduction in pain at the end of the 90-day trial when the affected joint was palpated. Vertical peak force was the key parameter measured to determine weight bearing of affected limbs. There was no significant change in mean peak force over the duration of the 90-day feeding trial for the control group. The mean vertical peak force increased significantly for the test group over the same time interval (TABLE 3). The percent mean change in vertical peak force was also significantly different between groups, indicating that the test group increased weight bearing on the affected limb over the course of the study. Additionally, only 31percent of dogs in the control group had improved weight bearing after the 90-day feeding trial, whereas 82 per cent of dogs in the test group increased weight bearing; this difference was also statistically significant (FIGURE 3).

TABLE 3

	Day 0	Day 90	Mean change	% Mean change
Control	72.8	72.6	-0.174	-0.58
Test	9.5	73.2	+3.71	+5.35††

Table 3. Canine force plate gait analysis vertical peak force† - summary.

† %BWkg; †† p=0.04, (Cross AR, et al. unpublished)

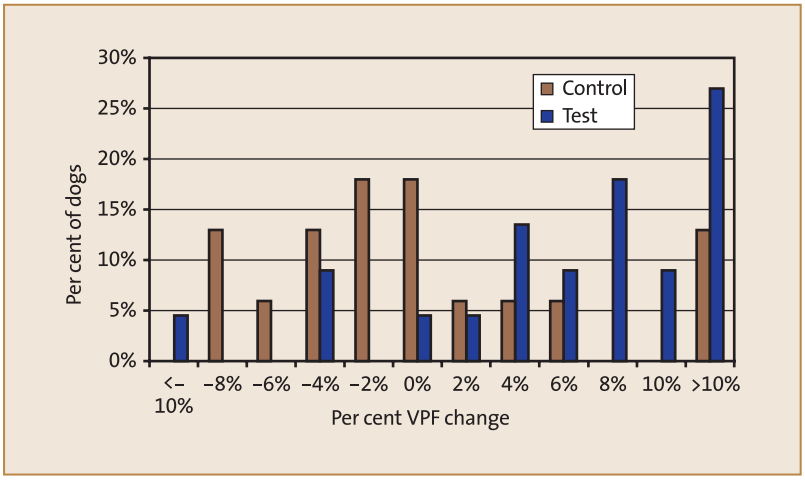

Figure 3. Effect of foods on vertical peak force (VPF) frequency distribution.

CONCLUSION

These clinical studies indicate that nutritional management using a therapeutic food with high levels of omega-3 fatty acids, and in particular EPA, helped improve the clinical signs of OA in dogs as measured by pet owners, clinical orthopaedic examination, and gait analysis of ground reaction forces.

KEY POINTS

The objectives of treatment for osteoarthritis are multifaceted, including:

- Reduce pain and discomfort
- Decrease clinical signs
- Slow the progression of the disease
- Promote repair of damaged tissue
- Improve the quality of life.

Current treatment modalities used to manage dogs with chronic pain caused by osteoarthritis include:

- Anti-inflammatory and analgesic medications
- Disease-modifying osteoarthritis agents
- Nutraceuticals
- Weight reduction
- Low-impact exercise programs
- Physical therapy
- Dietary management

Frequent, mild, weight-bearing exercise over an extended period has been shown to help patients with osteoarthritis to:

- Reduce body weight
- Increase joint mobility
- Reduce joint pain
- Strengthen supporting muscles

Diets designed for companion animals with osteoarthritis need to:

- Supply nutrition that is appropriate for the animal's age
- Contain specific nutrients that may help reduce inflammation and pain
- Provide the building blocks for cartilage repair
- Slow the degradative process
- Compliment the prescribed medications
- Provide tangible improvement in clinical signs.

Recent discoveries in fatty acid nutrition have provided clear evidence that canine osteoarthritis may be very responsive to dietary addition of specific fatty acids.

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