

ABSTRACT

GRUEN, MARGARET ELIZABETH. Qualitative and Quantitative Assessment of Pain in a Naturally-Occurring Model of Feline Chronic Pain (Degenerative Joint Disease). (Under the direction of Dr. B. Duncan X. Lascelles).

Degenerative joint disease (DJD) has a remarkably high prevalence in cats; nearly 92% may have radiographic evidence of disease, with approximately half of those having associated pain and mobility impairment. Given this high prevalence, it is surprising that research into this disease is in its relative infancy, with the first comprehensive and systematic studies taking place only in the past decade. Growing interest in treatment options for DJD and associated pain is driving forward research in this field. Further, there is burgeoning recognition that cats and dogs with DJDs represent important translational models of arthritides in humans, heightening the importance of better characterizing the behavioral and biological manifestations of DJD in cats.

We began by exploring the most fundamental issue – access to the cats and their owners for clinical and translational research studies. Through surveys of cat owners and veterinarians, we identified clinical trial features that facilitate or discourage owner and veterinarian participation. We found that recommendation from their veterinarian was important to cat owners in considering participation in a clinical trial, and that belief in the value of the study and engagement with the study's findings were important to veterinarians in recommending a clinical trial.

Next, we set out to quantify the caregiver placebo effect in trials of analgesics for cats with DJD, an effect that has stymied development and evaluation of potential therapeutics, and to develop relevant mitigation strategies for this effect. Using available data from a majority of placebo-controlled studies performed with client-owned cats with DJD, we found that the caregiver placebo effect was remarkably high, with 68% of owners rating cats as improved on subjective outcome measures while cats were receiving a placebo. We then focused on refinements to outcome measures and study designs to mitigate this effect. We found that the use of deterioration, a return of clinical signs following cessation of an active treatment, could be an effective measure of treatment efficacy against a placebo, and this provided a novel approach to our study design.

We next focused on the objective measures of accelerometry and soluble biomarker development. We applied the relatively new method of functional data analysis (FDA) to data from accelerometers worn by cats. Using FDA, we were able to show that differences exist in the pattern of activity shown during a 24-hour day by cats with DJD as compared to those without DJD, and that this pattern was significantly affected by age and orthopedic pain score. Further, we demonstrated that cats in homes exhibit a bimodal pattern of activity, with different patterns on weekends and weekdays. Further work to apply FDA to the analysis of treatment effects for analgesics is underway.

We then measured serum cytokine and chemokine concentrations in 186 well-phenotyped cats with and without DJD and associated pain using a multiplex panel of 19 different cytokine/chemokines. We found that individual analytes, in particular IL-4, IL-8, IL-

2, and TNF- α were associated with radiographic DJD score and orthopedic pain score. The strongest findings were for IL-8, where increased concentrations of IL-8 were associated with increased DJD score and increased pain score, although, the association of increased IL-8 concentration with increased age and the increase in both DJD score and pain score that occur with age may confound this relationship. While no single or group of analytes was able to reliably differentiate cats with and without DJD, this investigation was the first of its kind to assess the potential for a soluble biomarker associated with DJD or DJD-associated pain in cats.

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Qualitative and Quantitative Assessment of Pain in a Naturally-Occurring Model of Feline
Chronic Pain (Degenerative Joint Disease)

by
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DEDICATION

This dissertation is dedicated to my family for their love and support.

BIOGRAPHY

Margaret Gruen is originally from Hyde Park, in Chicago, Illinois. She attended Haverford College, in Haverford, Pennsylvania and majored in psychology (biological sciences concentration), with a project focused on swim stress analgesia in rodents. After college, she moved to Boston, Massachusetts to complete pre-requisites for veterinary school and worked for a biotech firm studying chemotherapeutic agents in rodent models of cancer. She then took a position as a nursing supervisor in the ER/ICU at Angell Memorial Animal Hospital. She returned to Illinois to obtain her veterinary degree, and then came to North Carolina State University where she has remained through an internship, residency in Animal Behavior, and clinical and research faculty positions in the Department of Clinical Sciences. She is boarded in Veterinary Behavior through the American College of Veterinary Behaviorists, and has completed a Masters in Veterinary Public Health at North Carolina State University.

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Chapter 1 : Introduction – An Overview of Degenerative Joint Disease in Cats

Introduction

A 2011 Institute of Medicine report disclosed that the economic cost of persistent pain among people in the US (\$600 billion annually) is more than for cardiovascular disease (\$300 billion) and cancer (\$250 billion) combined.¹ Despite these expenditures, numerous reviews have highlighted a crippling failure to translate basic research into new approved therapeutics for human pain. One reason for this shortcoming is the over reliance on rodents for drug efficacy studies. Spontaneous painful disease in companion animals has been recently noted for its translational potential since it may better reflect the complex genetic, environmental, temporal and physiological influences present in humans. However, for a spontaneous disease model to successfully contribute to translational research, the model must be well developed, including outcome measures reflecting the complexity of the disease in humans. The work described in this thesis contributes to the development of companion animals as suitable models by describing the development of outcome measures in spontaneous degenerative joint disease (DJD)-associated pain in cats. This thesis also provides critical new data that will enhance the ability of veterinarians to manage pain in their patients.

Global issues associated with veterinary pain research

DJD is the most common cause of chronic pain and mobility impairment in cats, yet our ability to measure this pain and associated mobility impairment remains lacking. The presence of DJD is associated with changes in activity, social behavior, and temperament in cats,²⁻⁶ and the prevalence of DJD increases with increasing age.^{7,8} DJD in cats likely results from a combination of genetic, metabolic, and biomechanical factors, similar to osteoarthritis

(OA) in people,⁹ though knowledge in this area of comparative medicine is still developing. Despite increased veterinary awareness of DJD, its diagnosis still lags behind the documented radiographic prevalence,⁷ in large part due to the difficulty of measuring pain, and the absence of a reference-standard for diagnosis. There are no known effective treatments for DJD-associated pain in cats, and no treatments are approved for use in the cat in the United States. This deficiency reflects in part our inability to measure pain and mobility impairment in cats.

Measurement of pain in any species is difficult, stemming from the multi-dimensionality of pain, which has both neurophysiologic and affective components. This multi-dimensionality in people includes cognitive, affective, behavioral, physiological, sensory, and socio-cultural domains¹⁰ (Figure 1). In humans, pain is what the individual reports as pain, and most emphasis has been placed on self-reports. However, there are individual differences in pain thresholds, and pain reporting is affected by context.¹¹

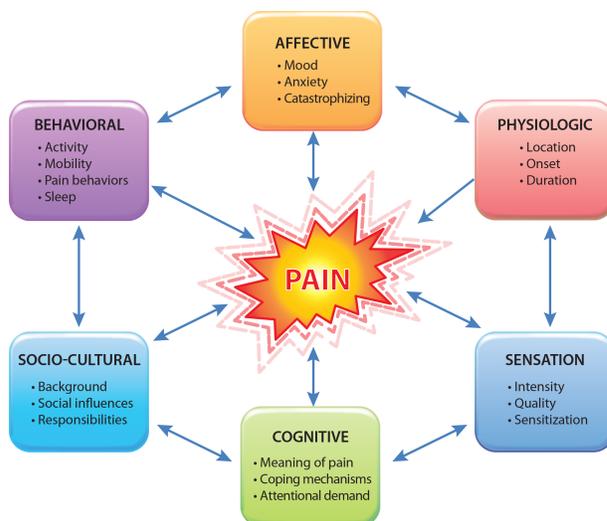


Figure 1. Multi-dimensionality of pain (adapted from (10)).

In veterinary species however, we are unable to rely on self-reports and must instead observe the behavioral, infer the affective, and partially quantify the physiological and sensory domains. In contrast to the emphasis on self-reports of pain in humans, the measurement of pain in non-verbal humans and other species relies on proxy measures by observers, and measures of one or more of the dimensions affected by pain. In cats, measurement of pain is particularly difficult given the relatively poorly understood species-specific behaviors and limitations in our detection and interpretation of signs that may be associated with pain. Our ability to “ask the cat” how it feels is limited, measured only indirectly via functional evaluations or by proxy report. It is commonly mentioned that cats, as mid-level predators, mask signs of pain and illness.¹²⁻¹⁴ While likely a contributing factor to the difficulty of assessment, we would argue that this explanation is overstated, and that as our awareness and understanding increase, so will our sensitivity to detect changes in behavior associated with pain. In developing methods to assess pain in cats, we can learn from but need to resist being confined by preconceived ideas about suitable or expected outcomes based on experience in other species. Despite these limitations, there is a convergence of evidence that DJD is associated with pain in cats, with owner-noted changes in behaviors – among them the inability to perform certain activities, and behavioral responses consistent with pain during veterinary examination.

The treatment of this pain is complicated by an incomplete understanding of the neurobiological basis of the generation of naturally occurring joint pain and the relative contributions of gain or loss of function at all neural levels in both the pain sensing system

and the endogenous analgesic systems. The reader is referred to the appendix (Appendix 1) for a brief overview of current knowledge on the biology of pain in DJD.

The approach to assessment that has been taken in dogs with joint pain, and that appears to have been relatively successful, is a combination of optimizing the awareness of pain, behavioral measures by a proxy (owner or veterinarian) and various quantitative measures of mobility such as measuring limb use. This triad approach – awareness, subjective and objective measures of mobility – is a reasonable initial approach to consider in the cat. This approach is based upon a realization of the multidimensionality of pain, and exploits the ability to measure different dimensions impacted by pain, even if the experience of pain itself cannot be measured in non-verbal species.

Just as in any species, measurement of pain associated with DJD in cats requires awareness of the presence and prevalence of the disease among veterinarians and owners, and diagnostic tools for evaluation of pain, mobility impairment, and alterations in the ability to perform activities. Strides have been made in increasing awareness among veterinarians, with a number of recent publications on the clinical importance of DJD in cats.¹⁵⁻¹⁸ Still, there remains an educational opportunity; one recent report found that only 60% of third-year veterinary students were aware that cats could develop DJD, despite 80% being cat owners.¹⁹ While as yet unstudied, the lack of awareness among cat owners in this population is likely generalizable to the wider cat owning population, though awareness is probably even lower among cat owners with no veterinary training. Reasons for this lack of awareness are many and include an attribution of signs of DJD to normal aging and the lack of “classical” signs of

DJD (such as limping, as observed in dogs and horses) in the presentation of most cats with DJD.²⁰ A further reason for the lack of awareness is the paucity of available treatments for DJD-associated pain in cats – marketing of effective treatments would include educational materials designed to increase recognition of the disease among owners. This, however, remains a circular issue, as development of treatments requires awareness of the need among cat owners and veterinarians, and depends, *critically*, on suitable outcome measures.

Beyond awareness, the successful development of a research program to study DJD, associated pain, and test therapeutics in cats depends on two central factors: 1) access to cats with DJD, and 2) outcome measures (both subjective and objective) that distinguish between cats with and without DJD and that reflect cats' response to therapeutic interventions. Clinical trials with owned cats are feasible; several have been successfully performed,^{3,21-26} however, low recruitment rates, common in human clinical trials, are a problem in veterinary clinical trials as well. No work has been performed in veterinary medicine to understand the barriers to participation in clinical trials. Current outcome measures lack either discriminative responsiveness (between placebo and active) or clinical applicability. The lack of responsiveness, particularly in subjective outcome measures, is due in large part to an overwhelming caregiver placebo effect, necessitating refinement of outcome measures and restructuring of study design. Further development of clinically applicable objective outcome measures for cats is needed to validate the subjective assessments and facilitate collection of reproducible and reliable results. Given the difficulty in assessing pain in cats, biomarkers indicative of DJD associated pain would fill a critical need in understanding this disease.

DJD in cats: Our current understanding

To contextualize the scope of DJD in cats, this dissertation will begin with a review of the prevalence of DJD in cats, followed by a review of current knowledge on the pathophysiology of DJD in cats, clinical signs and diagnosis, and outcome assessments.

A note on terminology

Studies on this topic in cats have referred to either “degenerative joint disease” or “osteoarthritis”. Though both terms have been applied to the disease in cats, frequently authors or groups will prefer one or the other, resulting in inconsistency in the terminology. Osteoarthritis (OA) refers to non-inflammatory pathologic changes in synovial joints that may be primary/idiopathic, or secondary to trauma or abnormal developmental features. DJD is an umbrella term, encompassing OA and inflammatory arthropathies of synovial joints, and degenerative changes to fibrocartilagenous joints, and also includes spondylosis deformans of the vertebral column.²⁷ For the purposes of this dissertation we will use the terms DJD and DJD/OA because, as discussed later, there is little information on the etiology of DJD in cats, and on the histological characteristics, making it difficult to sub-categorize the disease. Additionally, many studies have included cats with pathology in both synovial and fibrocartilagenous joints and spinal segments. However, when discussing individual studies, the terminology used by the authors of those studies will be used in this review.

Prevalence of DJD in cats is high

Radiographic evidence

The earliest studies investigating prevalence in cats were retrospective studies that reported evidence of DJD/OA in convenience samples of radiographs taken of cats for a variety of indications. Joints that were included in available views were evaluated for the presence or absence of DJD/OA based on radiographic features characteristic of OA in dogs, including the presence of osteophytes, enthesophytes, and subchondral sclerosis.²⁸ Results from these studies varied widely, ensuing from differences in available radiographs, and in definitions and criteria applied for describing both the presence and the severity of DJD/OA. The first report, by Hardie et al,²⁰ built upon a preliminary survey in cats²⁹ and evaluated radiographs from 100 cats over 12 years of age. Both the location and the severity (graded 0-3, with increasing severity) of radiographic features consistent with DJD were recorded. That study reported an overall prevalence of 90%, with only 10 cats having no radiographic evidence of DJD. When parsed as appendicular vs. axial, 10 of the 100 cats had evidence of DJD in the appendicular joints only, 26 in the axial skeletal segments only, and 54 in both the appendicular joints and axial skeleton. The majority of the affected joints/spinal segments were scored as relatively mild (scores ≤ 1); the elbow was the most severely affected joint, with 17% being scored a 2 or 3 and the lumbosacral articulation was the most severely affected spinal segment.

Two retrospective studies followed the report by Hardie et al., both also using a convenience sample of available radiographs. The first, by Godfrey et al.,³⁰ examined radiographs from 292 cats greater than 1 year of age, and reported a 22% prevalence of

DJD/OA (defined as increased subchondral bone density or periarticular new bone). Thirty-three percent of these reportedly had “clinically evident” DJD/OA, with a notation in the medical record of mobility impairment. This study also reported that the elbow was the most common site of DJD/OA, making up 55% of the affected joints. A study by Clarke et al. from the same year reported a 33.9% prevalence of DJD in radiographs from 218 cats.³¹ In the Clarke et al. study, both appendicular and axial skeletal DJD were identified and scored as mild, moderate, or severe, with a fairly even distribution between cats with appendicular DJD only (39.2% of affected cats), axial skeletal DJD only (32.4%), and both appendicular and axial DJD (28.4%). In contrast to previous studies, the coxofemoral joint was the most commonly affected site, representing 51% of the affected appendicular joints, while the elbow represented 26.5%. However, when a more permissive definition of DJD was employed, including enthesiopathy and peri-articular soft tissue mineralization as indicative of DJD, the elbow then represented 42% of affected appendicular joints, while the coxofemoral joint represented 34.2%. For all of these retrospective radiographic studies, it must be noted that a convenience sample of joints was used; only those that appeared in the radiographs available could be evaluated. Elbow joints are frequently seen in radiographs taken of the chest, and were typically overrepresented in these studies; more distal joints may not be routinely imaged. With the wide range of reported prevalence, while these early studies helped bring needed attention to the issue of DJD in cats, more systematic studies were needed to fully evaluate prevalence and severity, as well as identify risk factors for development of disease.

Two independent groups performed the first comprehensive, prospective studies on the prevalence of DJD in cats in 2010.^{7,8} Each of these studies evaluated 100 cats, however slightly different selection criteria led to marginally different results. In the study by Lascelles et al.,⁷ 100 cats (25 from each of 4 age groups) were randomly selected from a database of 1640 cats seen by a single feline practitioner. Cats were evaluated with a physical, orthopedic, and neurologic exam, CBC, serum biochemistry, and urinalysis. Cats were then sedated and orthogonal radiographs taken of each appendicular joint and spinal segment in order to evaluate the presence and severity (graded 0-10, with increasing severity) of DJD. This study found that 91% of the cats, across all age groups, had at least one site of appendicular DJD, while 55% had at least one site of axial skeletal DJD. Joints were commonly affected bilaterally; the percentage of cats with bilateral involvement ranged from 40% for the shoulder to 79% for the hip (excluding the manus and pes where prevalence was 0 – 1%). While severity scores for individual joints were typically low, this study suggested that DJD in cats was more widespread than previously appreciated. Age was found to be significantly associated with an increase in total DJD score (the sum of scores for each individual joint in a cat), but even in the lowest age group (cats > 0-5 years of age), 80% of cats had at least one affected appendicular joint. Prevalence of DJD in the axial skeleton increased across the age groups, from 16% in the lowest age group to 96% in the highest age group (cats 15-20 years of age). In order of decreasing frequency, the most affected joints in this study were the coxofemoral, stifle, tarsus, and elbow, however severity scores were highest for the elbow (median score=3). This shift in distribution of affected joints from earlier studies may be due to the finding of more severe scores for the elbow (making disease

in this joint stand out), as well as overrepresentation of views including the elbow in previous studies.

The second study, performed by Slingerland et al.⁸ similarly investigated 100 cats, though inclusion criteria were restricted to cats ≥ 6 years of age and relied on a convenience sample of cats presenting to the hospital rather than a randomly selected sample. This study employed a more conservative definition, focusing on OA and excluding the axial skeleton from consideration. The authors reported that 61% of the cats had radiographic evidence of OA in at least one joint, while 48% had involvement of more than one joint. When analysis was restricted to cats ≥ 14 years of age, the prevalence increased to 82% of cats with at least one affected joint. In 46% of the cats, joints were affected bilaterally. As in the study by Lascelles et al., both prevalence and severity increased with age. However, joints most commonly affected in the Slingerland et al. study were the shoulder, elbow, coxofemoral joint, and tarsal joint. In general, severity scores (scored as none, minimal, moderate, or severe) were low for all joints, with the elbows having the highest percentage of affected joints being scored as severe. This study excluded meniscal mineralization as indicative of OA, resulting in a lower prevalence of disease in stifle joints. The significance of meniscal mineralization in cats is discussed under pathophysiology below. In a follow-up study of spinal DJD conducted by the same group, radiographic evidence of spondylosis deformans was found in 39.4% of the cats examined, with increased prevalence associated with age. The most common site of degenerative changes was found in the T4-T10 region, however the most severely affected region was in the lumbosacral spine, consistent with other reports.^{20,31}

These two cross-sectional studies form the basis for our current knowledge about the prevalence of DJD in cats. While each reported somewhat different results, both studies found a strikingly high prevalence of radiographic DJD/OA in cats, particularly those over 10-14 years of age, highlighting the need to better understand the clinical signs associated with radiographic signs, and determine the underlying pathophysiology. Of note was the high percentage of bilaterally affected limbs, which can impact clinical signs that are discussed in a later section. Importantly, based on studies in cats showing low concordance between radiographic DJD and orthopedic pain scores, reported radiographic prevalence likely overestimates the number of cats with associated pain.²⁴ However the interpretation of radiographic changes is quite complex, and the clinical significance of radiographic evidence of DJD is unknown. It is likely that clinical significance varies by affected joint as well as by features of individual cats including number of joints affected. Additional features affecting clinical signs, including individual cat temperament, have been suggested³² but require further study. For the studies discussed in this thesis, radiographic DJD was part of the decision criteria for eligibility of cats.

Etiology and pathophysiology of DJD in cats

Understanding the underlying pathology and etiology of DJD/OA in cats is an area of active research. One early report postulated that ‘wear and tear’ or primary DJD/OA was less likely in cats than in dogs, due to the sedentary lifestyle of most cats,³³ and it was speculated that it was likely that DJD/OA in cats was secondary to undetermined factors, rather than being a primary disease.²⁰ It is likely, as in other species, that the etiology of DJD/OA will vary from joint to joint, and between individual cats. However, reports investigating the

cause of DJD/OA have failed to uncover an underlying cause for DJD/OA, supporting DJD/OA as generally a primary disease in cats, and leading some authors to term it a “constitutional disease”.⁸ Exceptions include breed specific disorders such as mucopolysaccharidosis type VI (a storage disease resulting in abnormalities in cartilage and bone) primarily found in Siamese cats,³⁴ and osteochondrodysplasia in Scottish Fold cats.³⁵ Additional underlying causes of DJD in cats are postulated to include congenital, post-traumatic, nutritional, and infectious/inflammatory¹⁶ but these likely represent a minority of cases. In the prevalence study by Godfrey et al.,³⁰ an underlying cause for the OA was only ascribed to 11% of the affected cases, with causes including bilateral hip dysplasia, a fractured femoral condyle, osteosarcoma, and acromegaly. Clarke et al.,⁴ ascribed a primary underlying cause to only two of 28 (7%) cats with OA, with an additional six having a combination of primary and secondary OA. Three of these eight cases were listed as post-traumatic, while seven were diagnosed with hip dysplasia (two cats had both hip dysplasia and post-traumatic OA).

The contribution of hip dysplasia to the development of DJD/OA in cats is not as clearly understood as it is in dogs. In dogs, hip dysplasia, particularly increased passive laxity measured by distraction index, appears to increase the probability of development of DJD.³⁶ The Norberg angle, a measure calculated from ventrodorsal radiographs of the pelvis, is often used to evaluate hip laxity. The Norberg angle is greater in cats than in dogs, even in non-disease state.³⁷ While hip dysplasia affects cats they seem to have and tolerate greater laxity when compared with dogs.³⁷ However, despite tolerance of higher laxity, hip dysplasia likely is associated with DJD in cats.³⁷

Joint pathology has been investigated in cats. Macroscopic evaluation of cartilage integrity has been performed for the elbow, tarsus, stifle, and coxofemoral joints,³⁸ while concurrent histologic characterization of joints affected by naturally-occurring DJD in cats has been limited to the elbow^{27,39} and stifle⁴⁰ joints. In a study by Freire, et al.,³⁸ macroscopic evaluation of the joints from 30 adult cats (mean age of 12 years) showed cartilage damage in 72% of elbow joints, 53% of tarsal joints, 80% of stifle joints, and 73% of coxofemoral joints. Evidence of DJD was detected radiographically in 42%, 57%, 65%, and 49% of these joints, respectively. Further investigation of the elbow showed that damage ranged from fibrillation to complete effacement of the articular cartilage with exposure of the subchondral bone. The most severe damage was in the medial compartment, at the medial coronoid process. Histologic damage to the humerus was similarly most severe to the medial aspect.^{27,39} Contrary to findings in dogs, where elbow OA is frequently associated with a fragmented medial coronoid process, this pathology was not found in cats.³⁹ Evaluation of the stifle joint of these cats showed that meniscal mineralization, apparent on radiographs, was characterized by mineralization and ossification of the cranial pole of the medial meniscus, and significantly associated with cartilage damage to the medial femoral and medial tibial condyles.⁴⁰ As these were cadaveric studies, correlations with clinical signs are not known. Collectively, in addition to lending understanding to pathology at the level of the joint, these reports highlight the dramatic damage that can be present, even in the absence of radiographic evidence of DJD.

Contributions of indoor/outdoor status, early neutering, diet, and obesity on DJD/OA development in cats are not well understood. One study on the long-term effects of early

neutering in cats did consider “arthritis” among potential outcomes investigated, and no association was found. However, the diagnosis of arthritis depended on owner report, and the overall prevalence was surprisingly rare (<1%).⁴¹ This study is likely better used as an example of the lack of owner awareness of DJD, and further work on the epidemiology of DJD in cats is needed. While obesity has been shown to be associated with OA in dogs⁴² and humans,⁴³ this association has not been well established in cats. In the prevalence study by Lascelles et al.,⁷ body weight was not significantly associated with either the presence or severity of DJD, but this may be due to the strong relationship between DJD score and increasing age, and the tendency for many cats to lose weight with age. However, one study did show that heavier cats were more likely to be taken to veterinarians because of lameness, compared to healthy weight cats.⁴⁴ Caloric restriction over years (lifetime) leads to decreased incidence of radiographic evidence of OA in dogs,⁴⁵ but similar longitudinal work has not been performed in cats and is needed. In attempts to find simple, unifying explanations of the etiology of DJD in cats, one study evaluated gene alterations measured in 29 cats with DJD and found that, compared with controls, cats with DJD had down-regulation of genes involved in immune system pathways that warrant further study.⁴⁶ These genes were differentially expressed even when controlling for age.⁴⁶ Another study evaluated whether exposure to *Bartonella* was associated with DJD in cats, and surprisingly found that cats with higher radiographic DJD scores and cats with higher orthopedic pain scores were less likely to be seropositive for three *Bartonella* species than cats with lower scores.⁴⁷ Implications from these two studies need further investigation, including longitudinal and mechanistic perspectives, but suggest that immune system regulation is involved in the manifestation of DJD in cats.

Veterinary diagnosis of DJD-associated pain in cats relies on convergent evidence from examinations

For veterinarians, diagnosis of painful DJD/OA currently requires a combination of owner-noted mobility impairment, findings on physical and orthopedic exam, and evaluation of painful joints through imaging. Additional assessments, including pressure walkways and fluoroscopic imaging, have been developed,⁴⁸⁻⁵¹ but lack clinical feasibility and are discussed under outcome assessments. Routine clinical pathology findings are not typically abnormal, though many cats will have co-morbidities associated with aging including hyperthyroidism, chronic kidney disease, cardiovascular disease, and others. It has been recently shown that the overlap between chronic kidney disease and clinically relevant DJD (DJD with associated mobility impairment) is high, with 68% of cats presenting for analgesic therapy trials having concurrent chronic kidney disease.⁵² Other clinical pathology abnormalities that have been reported include positive associations between radiographic DJD score and urine pH, lipase, and cholesterol,⁷ however reasons for these associations are not known.

Physical exam findings

Physical exam findings may be generally normal in many cats with DJD. The most common finding in cats with DJD is muscle atrophy, particularly over affected limbs. As many cats develop bilateral disease, muscle atrophy may be present over both hindlimbs or both forelimbs. Cats with bilateral hindlimb atrophy develop a ‘torpedo’ like appearance, with narrowing of the hindquarters due to muscle atrophy (Figure 2). Other findings on physical examination may include areas of matting due to the inability to groom well, and

generalized lack of luster to hair coat, though these are not specific to DJD, and can be influenced by caregiver-cat grooming or diet.¹⁹



Figure 2. Cat with bilateral coxofemoral DJD and hindlimb atrophy.

Orthopedic exam findings

Orthopedic exams are performed in cats by palpation and manipulation (flexion and extension) of each appendicular joint, and application of pressure and mild manipulation along the spinal column; flexion at the lumbosacral joint is used to evaluate lumbosacral pain. At the NCSU Comparative Pain Research Laboratory, orthopedic exam findings are recorded using a standardized scale, although this has not been systematically evaluated for validity (Figure 3). Orthopedic exams serve to identify both areas with physical changes to the joints (thickening and effusion) and to assess for the presence and severity of pain. Cats with DJD may have evidence of joint thickening due to peri-articular mineralization, and crepitus or effusion may be present in one or more joints. Pain on orthopedic exam is reflected by a change in the behavior of the cat during manipulation of the joint.⁵³ Many cats

will respond with vocalization or aggression, or attempts to escape as severity of pain increases. Some cats, however, may instead display a decrease in vocalization and decrease in attempts to move or escape, emphasizing the varying responses seen, and underpinning that changes from baseline behavior are probably most important.

ORTHOPEDIC EXAM – Day 0

Performed by: _____
 If unable to perform exam, why? _____

R Forelimb	Pain (0-4)	Creptus (0-2)	Effusion (0-2)	Thickening (0-2)
Feet				
Carpus				
Elbow				
Shoulder				
R Hindlimb	Pain (0-4)	Creptus (0-2)	Effusion (0-2)	Thickening (0-2)
Feet				
Hock				
Stifle				
Hip				
L Forelimb	Pain (0-4)	Creptus (0-2)	Effusion (0-2)	Thickening (0-2)
Feet				
Carpus				
Elbow				
Shoulder				
L Hindlimb	Pain (0-4)	Creptus (0-2)	Effusion (0-2)	Thickening (0-2)
Feet				
Hock				
Stifle				
Hip				
Spinal Column	Pain (0-4)			
Cervical				
Thoracic				
T-L				
Lumbar				
L-S				

Key
 Pain scale based on palpation
 0 No resentment; normal amount of movement or wriggling
 1 Mild withdrawal; mildly resists
 2 Moderate withdrawal; body tenses; may orient to site; may vocalize / increase in vocalization
 3 Orients to site; forcible withdrawal from manipulation; may vocalize or hiss or bite
 4 Tries to escape / prevent manipulation; bite/hiss; marked guarding of area
 Creptus, Effusion, Thickening: none (0); slight-moderate (1); significant-severe (2)

Figure 3. Orthopedic examination score rubric. This rubric is used by the NCSU Comparative Pain Research Laboratory. Scores are assigned based on the quality of the reaction from the cat.

Evaluation of range of motion, either subjectively or objectively via goniometry can be a component of the orthopedic exam, and larger range of motion has been shown to be associated with a lower likelihood of DJD in the elbow, shoulder, and carpal joints^{31,32} but normal ranges for cats have not been described and goniometry may be more useful for ruling out DJD than for describing severity or staging the disease.

Functional or performance testing is an additional component of the orthopedic exam. In cats, gait analysis is often difficult, as the preponderance of cats do not walk on a leash or willingly trot in unfamiliar surroundings. Many cats may be unwilling to adopt a normal posture and walk around an exam room freely, making it challenging to assess the fluidity of their movement. The frequently bilateral nature of DJD in cats makes overt limping or lameness an uncommon manifestation³ as changes in gait are distributed over affected limbs. As lameness is not a typical feature, gait analysis as part of a veterinary exam may be unrewarding, however cats may be encouraged to walk across an exam room by placing them at one end and their carrier or other hideaway at the other. Other functional tests that can be useful for cats include having the cat jump up (if the cats can be enticed to jump onto a chair or an owner's lap) and jump down from a chair or the exam table. Cats with DJD, particularly those with DJD in the hindlimbs, may hesitate prior to jumping up, or may not be able to "clear" a jump, with the dorsal aspect of their hindpaws hitting the edge of the surface they are attempting to jump on to. They may also use their forelimbs to help pull themselves up. Cats with DJD in their forelimbs often reach down prior to jumping down, or may land on their forelimbs followed more quickly than normal by their hindlimbs, presumably to shorten the distance of the jump and lessen the impact/force placed on their forelimbs when jumping down. Cats with forelimb DJD-associated pain are also described as having heavy or harsh landings.

A recent attempt was made to develop a systematic tool for use in veterinary examination of cats with DJD/OA.¹⁹ Colony-housed cats with and without OA were evaluated on exploratory behavior, body posture, gait/locomotion, interactive behavior, body

condition, and palpation using a numerical rating scale. In pilot work (n = 11 cats), only gait was able to distinguish between OA and non-OA cats, while in the full study (n = 29 cats), no scale item reliably discriminated between groups. Importantly, palpation scores were actually higher (worse) for non-OA cats. Given the likely increased difficulty in evaluating gait in client-owned cats, where unfamiliarity with the veterinary clinic can inhibit normal exploratory behaviors for many cats, this scale will require further refinement prior to clinical application. However, it represents important work that highlights the complexities of assessing pain in cats.

Diagnostic imaging

Diagnostic imaging is often used to corroborate findings on physical or orthopedic exam. Over 15 years ago, the radiographic evaluation of joints in cats was reviewed⁵⁴ with the common pathologic findings in cats suggested to include periarticular and intraarticular mineralization, enthesophytosis, osteophytosis, subchondral sclerosis, and ventral or dorsal spondylosis along the vertebral column. Further detail on the radiographic findings, and in particular the differences between dogs and cats, was recently communicated in a review¹³. In general, radiographic evidence of DJD is similar to that in dogs, however awareness of species-specific differences in presentation is important in the interpretation of radiographs, and an educational imperative for veterinarians. For example, while dogs with OA of the coxofemoral joints typically show more remodeling of the femoral head and neck, for cats, a more common presentation is osteophytosis along the craniodorsal acetabular margin, with less remodeling of the femur⁵⁴ (Figure 4). In addition, as discussed above, meniscal mineralization is reported more commonly in cats than in dogs.^{13,38,54} If we consider cartilage

damage (measured via India ink retention) as indicative of true joint disease, then radiographic sensitivity is only moderate, detecting joint disease in 53% of elbows, 60% of coxofemoral joints, and 68% of stifle joints. Specificity of radiographic signs is higher for the elbow (88%) and coxofemoral (81%) joints, but is lower for the stifle (50%).³⁸

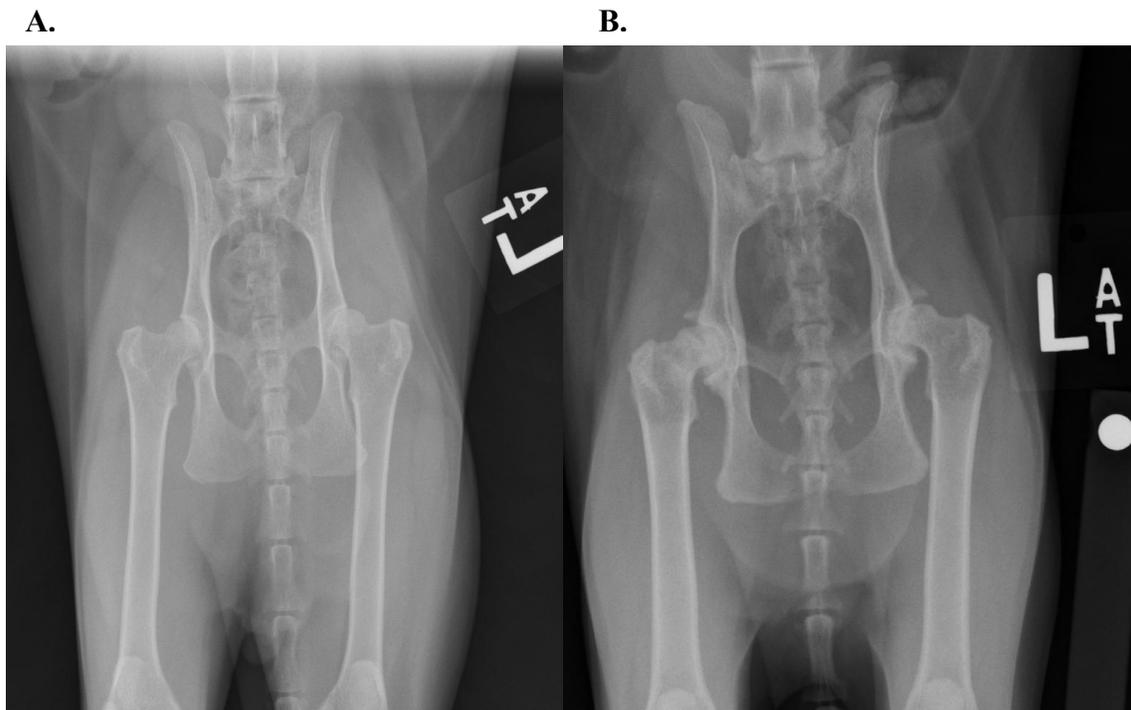


Figure 4. Radiographs of coxofemoral joints (ventrodorsal projection) from a normal cat (A) and a cat with severe bilateral coxofemoral DJD (B). Note the presence of osteophytes on the cranial acetabular edge and osteophytosis and thickening of the femoral neck in the radiographs from the affected cat.

Magnetic resonance imaging (MRI) is commonly used in humans for evaluation of joint disease and structural changes resulting from OA. The cost of this imaging, and the need for general anesthesia to perform MRI in untrained companion animals has limited its diagnostic applications for DJD/OA in veterinary medicine. Preliminary work investigating

coxofemoral OA in cats (n = 4) has shown that MRI can detect osteophytes, sclerosis, and bone marrow lesions, and may be more sensitive than radiography for this joint, but comparative sensitivities have not been rigorously evaluated.^{48,55}

An additional imaging modality, thermographic or infrared imaging, has been partially evaluated in cats with DJD. Thermographic imaging uses an infrared camera to visualize changes in superficial temperature, on the premise that inflammation in deeper structures (including joints) will be reflected in these surface readings.⁵⁶ However, further work with this method is required; initial investigation failed to show strong evidence of agreement between palpation, owner evaluation, and thermographic imaging, and was not evaluated against radiographic images.⁵⁷

Arthrocentesis

Evaluation of synovial joint fluid is a potential further diagnostic tool, however the sensitivity of this measure to detect DJD is low. Synovial fluid from joints affected by OA typically has low cell counts, though low-levels of reactivity may be seen in mononuclear cells.^{58,59} While synovial fluid analysis can be useful for ruling out inflammatory or infectious arthropathies, the balance of the value of this diagnostic test with the difficulty of obtaining synovial fluid from cats suggests it should be reserved for cases with a high clinical suspicion of infectious or inflammatory disease, and one or more joints with effusion. Synovial fluid may prove more valuable for biomarker development,^{60,61} but this has not been evaluated in cats.

Limitations of Veterinary Diagnostics

It is widely recognized that signs of pain are difficult to assess in cats,^{13,62} therefore orthopedic examination findings should be considered in context of other signs including owner noted mobility impairment, muscles loss, and results of imaging. Responses consistent with pain, particularly if repeatable, should not be ignored if radiographic evidence of DJD is absent, as the concordance between radiographic signs and pain is marginal. For example, in one study, orthopedic examination pain scores in cats with and without meniscal mineralization were not significantly different,⁴⁰ but pain scores obtained during manipulation of elbow joints is predictive of radiographic evidence of DJD.³² While orthopedic exam pain has shown a higher predictive value for presence of radiographic DJD than crepitus, effusion, or thickening of the joint, pain and radiographic DJD were found in only 0-55% of the joints with radiographic DJD (varied by location).³² However, the *absence* of pain, effusion, and crepitus on orthopedic exam has high specificity and negative predictive value for radiographic signs.³² While several authors have stated that pain appears to be present in some joints without radiographic signs of DJD^{13,32} when performing studies of DJD-associated pain in cats, these cases cannot be included until there is some corroborating evidence that the pain is the result of joint pathology.

While much more is now known about how to evaluate cats with DJD, the nature of the relationship between findings on orthopedic examination, radiographs, and clinical signs remains incomplete and confusing. In the absence of clear diagnostic and treatment guidelines, DJD in cats will remain an under-diagnosed, and under-treated disease. However, as there are no currently approved therapeutics for the treatment of chronic pain in cats,

improved diagnostic criteria are a research imperative. Selection of appropriate cases for clinical trials is central to the successful development of treatments for cats with DJD.

Owner evaluations offer insight to clinical signs present in the home environment

Clinical signs of DJD – owner evaluations

With the limitations in the ability of veterinary evaluations to fully evaluate impairment associated with DJD, owner-noted behavioral signs from the cat's home environment are important to understand the clinical picture. This assertion is based on the fact that in other species, including dogs, DJD-associated pain results in alterations in mobility and the ability to perform activities. It also alters behaviors associated with mood, social interactions, and play. Owners of dogs with OA have been able to detect response to non-steroidal therapy over placebo, and are likely assessing features separate from those measured by kinetic analyses.⁶³

Two approaches have been taken to understanding the behavioral changes owners may see in cats with DJD. The first is performed by having owners evaluate lists of behavioral signs and attributes, and determining which behaviors best discriminate between two extreme groups of cats, those without DJD and those with severe DJD. The second is accomplished by asking owners about behaviors that improve when affected cats are given an analgesic therapy, on the assumption that the behaviors that change in response to analgesic administration are those that were altered by the presence of pain. These two approaches have generated a list of behaviors that cats may display when affected by DJD. However, awareness of DJD and associated pain among cat owners is thought to be relatively

low, with many behavioral signs of pain being attributed to normal aging, rather than representing a pain state. Additionally, this approach suffers from the fact that the initial questions are based on preconceived perceptions about what behaviors may be altered in cats with DJD.

The two cross-sectional prevalence studies discussed above are examples of the first approach; each incorporated owner ratings of behaviors that could be correlated with radiographic findings. In both of these studies, behavioral signs associated with DJD/OA were also strongly associated with age, as radiographic evidence of DJD/OA was strongly associated with age. This helps us understand why owners would attribute behavioral signs to aging, but does not negate the need to provide treatments for cats with DJD. In the study by Slingerland et al.,⁸ the behavioral signs associated with DJD/OA included less jumping, decreased clearance of tall obstacles, stiffness, difficulty navigating stairs, less grooming, and elimination directly over the edge of the litter box. Other owner-rated changes associated with DJD/OA included a change in accessibility of the cat's favorite places, and a perception of a decrease in overall cat satisfaction [with life]. Only one of these behavioral signs, elimination directly over the edge of the litterbox, was not strongly associated with age, however not all cats with DJD/OA will display this sign.² The follow-up report that focused on cats with spondylosis deformans of the vertebral column noted that the presence of moderate to severe lumbosacral DJD was associated with more aggression toward owners in social interactions (decreased tolerance of petting, less likely to greet people, and less satisfaction with life).⁶⁴ The prevalence study by Lascelles et al.⁷ also found significant differences between cats with and without DJD in litterbox behavior (specifically that cats in

the high DJD group were less likely to cover their urine) and in sleeping behavior, with cats in the high DJD group sleeping more than cats without DJD. In addition, this study found significant differences between the groups for 15 other behaviors including walking, running, jumping up and down, ascending and descending stairs, playing with other pets, social behaviors and play, and ability to rise from a resting position and stretch.

The second approach, observing what behavioral signs improve with treatment, has been studied via owner survey² as well as in research study settings.^{3,62} Results of a survey of 50 owners of cats with OA found that the most common signs of OA recognized by owners included jumping, stair use, speed, agility/clumsiness, grooming, play/hunting, and gait (which included limping, stiffness, and changes in limb carriage or appearance). Changes in litterbox use (including elimination outside the box or different maneuvering inside the box) were present in ~40% of the cats. Those signs most responsive to analgesic treatment (unspecified) were jumping, stair use, speed, gait, activity level, and mood.² However, there were limitations to this study, most notably in the manner in which diagnosis of OA was made. In this study, 64% of the cats had a combination of owner-noted changes and physical exam *or* radiographic evidence of OA, 30% had only owner reported changes, with no physical examination changes and no radiographs performed, and 6% had only physical examination findings, with no owner reported changes prior to veterinary diagnosis. In several of the cats in each group, treatment with an analgesic was used to confirm the diagnosis. Despite the limitations, understanding what behaviors owners are observing, and those that they note are improved with treatment is important in developing outcome assessments for owners.

In a separate study with 28 cats, Clarke et al.⁶² found that owners rated their cats as improved in unwillingness to jump, reduced height of jump, and stiff gait following a 4-week course of a non-steroidal anti-inflammatory drug; seeking seclusion, vocalizing when handled, resentment of handling, and aggression when handled did not show significant improvement. Again, however, this second approach is also based on preconceived assumptions of what behaviors may change. A more important factor to consider in this second approach is the caregiver placebo effect, whereby owners may ‘see’ improvement when none is really present.

Regardless of the shortcomings, taken as a whole, these studies provide insight into the types of behaviors that may be altered, and that owners may be able to evaluate readily in their cats. Not only do these offer information for the development of standardized questionnaires, but also highlight the types of behaviors that can be featured in educational materials about DJD. The bulk of the behaviors are actions (movement, stairs, etc.) but owners are able to appreciate more intangible features such as mood.

Current outcome measures build on owner and veterinary evaluations

Subjective outcomes (owner)

Studies of behavioral signs noted by owners were used for the development of current owner questionnaires or clinical metrology instruments (CMIs). Several attempts have been made to generate CMIs that capture behaviors of cats with DJD, and are responsive to analgesic treatments; few of these attempts have undergone rigorous evaluation of validity. Of the CMIs that have been developed for cats with DJD, there is variation in the

measurement scale used (numerical rating scale, visual analogue scale, or binary choice). Responsiveness of the scales has typically been tested using a single non-steroidal anti-inflammatory medication, though there has been application in non-drug settings including diets²³ and nutraceuticals.⁶⁵

Many CMI's have been developed for use in a specific study, and only two CMI's for DJD-associated pain have been used in more than one clinical trial. These are the Client Specific Outcome Measure (CSOM) and the Feline Musculoskeletal Pain Index (FMPI). The Client Specific Outcome Measure was developed for use in dogs where it was termed the Cincinnati Orthopedic Disability Index⁶⁶ and then adapted for use in cats.²⁴ Its appeal is that the activities followed are individualized for each animal. It allows owners, working with a trained clinician, to select a set number of activities that *their* cat is impaired in performing, rate the amount of difficulty the cat has in performing those activities (typically on a scale from 0 = impossible to 4 = no problem), and then this personalized CMI is used to follow this set of activities over time and following administration of an analgesic treatment. Change from baseline in total score (the sum of scores for each activity) is used as the outcome of interest, with published criteria for success/failure also being employed.^{67,68} The Feline Musculoskeletal Pain Index was developed based on the results of the prevalence study by Lascelles et al., using the items that discriminated between cats with and without DJD,⁶ and underwent readability, reliability, and responsiveness testing.^{5,6,21} It is a questionnaire consisting of set questions, in contrast to the CSOM. A recent review of clinical metrology¹² instruments used for the measurement of pain in cats concluded that of the CMI's that have been developed for chronic pain, none show complete responsiveness (the ability to detect

change in response to an analgesic) or validation. Despite significant effort by researchers in this field, the current CMIs are generally able to distinguish between cats with and without DJD, but are not able to reliably show efficacy of therapeutic interventions. Certainly one feasible reason for this is that no truly efficacious treatments have been rigorously studied for cats, but a more likely cause is the profound caregiver placebo effect that exists when using proxy rating systems. This effect, and methods for refinement of CMIs and study design, are discussed in Chapter 3 of this dissertation.

Objective outcomes

In addition to the subjective outcomes, several objective outcome measures have been developed or adapted for use in cats with DJD/OA, generally based on approaches that have been successful in the dog. These include pressure walkway based evaluation of kinetic variables such as peak vertical force,^{19,48,49} and fluoroscopic evaluation of gait.⁶⁹ These objective measures of limb use and function demonstrate an ability to discriminate between cats with and without DJD/OA, particularly following exercise,^{48,49} but show poor responsiveness to a non-steroidal anti-inflammatory in laboratory-based studies⁴⁹ and have not been tested in a client-owned population of cats with DJD. Gathering such data in client-owned cats appears feasible, with two studies performed in client-owned cats reporting the ability to obtain reliable pressure-walkway results in 65%⁷⁰ and 81%⁷¹ of the cats tested. Based on work in laboratory cats, recent recommendations for kinetic data acquisition and analysis have been published.⁵⁰ Despite this information, the equipment required for these tests is expensive, acclimation of the cats is generally required,^{48,50} the applicability to client-

owned cats has not been tested, and therefore, at the moment, routine clinical use is not expected.

Central sensitization contributes to increased pain from a diseased joint through facilitated nociceptive transmission and increased gain (hypersensitivity) in the spinal cord and/or at higher levels of the neural axis. Quantitative Sensory Threshold (QST) testing is used to identify these somatosensory abnormalities. QST involves the application of mechanical, thermal or electrical stimuli to the tested area by the use of non-invasive techniques,⁷²⁻⁷⁴ with the implication that alterations of somatosensory function are due to peripheral, central, or a combination of peripheral and central changes in sensory information processing. In human OA, increased pain sensitivity in response to a variety of mechanical, thermal, and chemical stimuli has been reported in patients with OA at sites over symptomatic joints and remote from them.^{72,73,75} Up to 70% of human OA patients reportedly have somatosensory abnormalities,⁷⁶ and 20 to 40% of OA patients are thought to have a predominantly 'central' drive to the pain perceived – i.e., the pain being perceived is generated predominantly from the central nervous system rather than the joint, due to central sensitization.⁷⁷ This underscores the importance of sensitization in pain processing.

Recently, mechanical QST testing has been investigated in laboratory colony cats. Studies to date have shown that von Frey testing (mechanical QST testing) is able to discriminate between some cats with and without DJD (defined as the presence of both radiographic evidence of DJD and abnormal orthopedic exam),^{49,78} with approximately 25% of cats with DJD in one study showing responses consistent with allodynia (where non-

noxious stimuli are perceived as noxious).⁴⁹ Specific patterns of evoked temporal summation were also able to differentiate between cats with and without DJD.⁷⁸ However, as with the force plate and pressure walkway, widespread clinical applicability has not been demonstrated, and no studies have been performed in untrained, client-owned cats. As in humans, application of QST testing may be best suited to identification of subgroups within the population of cats diagnosed with DJD. Populations defined as allodynic may require different treatment regimens; cats categorized as allodynic, in contrast to cats with DJD without allodynia, failed to show responsiveness to a non-steroidal anti-inflammatory medication in one study.⁴⁹ While preliminary, the suggestion of measurable central sensitization in cats with DJD is in line with findings in dogs⁷⁹ and humans⁸⁰ with OA, and requires further research into outcome measure refinement.

The most commonly used objective outcome measure is accelerometry-based assessment of activity. Accelerometers are typically small, portable devices that are worn for set periods of time and record “activity” as counts per unit time (epoch length). Accelerometers have been used in studies of chronic pain in humans,⁸¹ dogs,^{82,83} and cats.^{22,49,84,85} In cats, the two accelerometers that have been used are the Actical® and the Actigraph®. Both of these accelerometer types can be mounted on a collar or harness, worn by cats in their natural environment, and are accepted as surrogate measures of cat activity.

In cats, the validity of this assumption was systematically tested by Lascelles, et al.⁸⁶ by comparing objectively measured distance moved (via analysis of video recordings using Noldus® Ethovision software) with the output from Actical® accelerometers worn on either

a collar or a harness. Reported correlation between harness and collar output was excellent ($R^2 = 0.95$) with good agreement between activity counts and distance moved.⁸⁶ This supported the use of accelerometry as an objective measure of activity in cats.

In addition, activity counts have been shown to increase in cats with DJD/OA in response to an analgesic, both in laboratory cats⁴⁹ and client owned cats.^{22,24} However, the use of activity counts has not been able to robustly distinguish between cats with and without DJD/OA due to high inter-individual variability,^{49,78} with the exception of one study in which overnight activity was shown to be lower in cats with DJD than cats without DJD living in a colony setting.⁴⁸ Activity counts have historically been used in either their raw form (activity count per epoch) or log-transformed, and then averaged over days or weeks to obtain a single value per cat. While this method allows for quantification of change over time, information about the pattern of activity is lost. Information about the pattern of activity is potentially very important. For example, overall activity in an individual may not change with analgesic treatment, but the resting periods may be more restful (decreased activity), and the active periods more active, with no-net change in ‘mean’ activity, but important changes in activity due to pain relief. Functional data analysis is a statistical method for analyzing patterns. This type of analysis allows one to understand features of the data that describe the pattern, as well as investigate covariates of interest (such as age, body condition score, etc.).⁸⁷ The application of functional data analysis to activity pattern data analysis in cats is explored in chapter 4 of this dissertation.

Finally, an as yet undeveloped objective assessment is a serum or plasma biomarker (or panel of biomarkers) associated with DJD and pain. In humans, there is great interest in the development of biomarkers for OA and associated symptoms.⁸⁸ Biomarker development focuses on imaging or biochemical markers that can contribute to understanding best methods for diagnosing the disease, quantifying the burden of disease, or fluctuating in response to treatment for OA.⁸⁸ Several hurdles remain, including determining the most appropriate sampling site, yet progress toward biomarkers in OA is being made.⁸⁸ With growing recognition of the inflammatory component of OA,^{89,90} several cytokines and chemokines are under investigation as potential biomarkers for this disease.

Given the difficulty in measuring pain in cats, an objective marker for DJD pain would be extremely desirable. Development of this field has been limited by the inability to accurately phenotype cats, and is also limited by the relative lack of feline-specific antibodies for testing. However, a feline-specific cytokine panel has been developed for multiplex testing, and access to this technology was available to us. With the suggestion of alterations in cytokine profiles in humans with OA and pain,⁹¹ and our sample bank of serum and plasma samples from cats (well-phenotyped in a standardized way as a result of the outcome measure development work), we have begun work to characterize cytokine profiles associated with DJD and pain in cats (discussed in Chapter 4).

Significance

This introduction has reviewed the current state of knowledge on DJD in cats, and highlighted the difficulty of measuring pain in cats. Currently, diagnostic confidence is based

on a convergence of clinical signs and veterinary findings. However, limitations exist within each type of diagnostic evaluation: findings from palpation potentially overestimate presence or severity of pain or are overly dependent on cat temperament; owner recognition of clinical signs is often poor and owner-scales are subject to a profound placebo response; and limitations exist in the utility and interpretation of currently available objective measures.

Addressing this difficulty in assessment of pain is critical, however, because not only does DJD affect a great number of cats, but also because joint disease in cats may be a valuable translational model for the study of OA and associated pain in people. The current OA research paradigm takes discovery in induced models of disease in rodents and applies this to naturally-occurring disease in humans.⁹² Shortcomings of this approach are numerous, but include lack of shared environment, lack of genetic diversity among rodents used in models, and short timelines from induction of disease to study endpoint. These shortcomings are likely partially responsible for the crippling lack of translation of basic research into new approved therapeutics for treatment of persistent pain, as highlighted in several recent reviews.^{93,94} There is a need for translational models with higher fidelity to humans in order to understand adaptive (or maladaptive) changes that occur over the lifetime of the disease.^{92,95} Indeed, the use of spontaneous painful disease in companion animals was highlighted as one of the changes that could be made to help improve translation of basic science to new therapeutics.⁹³

Deeper understanding of DJD in cats may, therefore, be advantageous to both human and cat health. Knowledge gained from the study of DJD in cats will benefit cats, and lend

understanding to features of joint disease in people that are not well addressed by the use of induced models. In particular, features such as risk factors for development of disease, neurobiological changes in pain transmission, and effects on spontaneous activity, may all be better studied in a naturally occurring model. This model also allows for an intermediate or parallel platform for testing efficacy of medications as depicted in Figure 5. Treatments for DJD and OA may be palliative, targeting only the associated pain, or disease modifying, and cats offer an opportunity for evaluation of both.

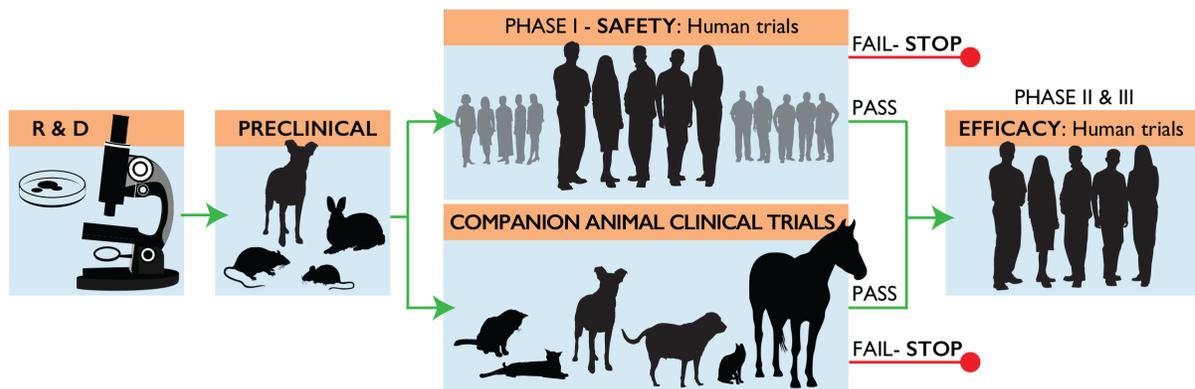


Figure 5. Suggested pathway for integration of naturally-occurring DJD into the development of treatments for both humans and companion animals.

The factors that limit development of a research program to study DJD and associated pain in cats are the same ones that limit full application of the feline model as a translational model. These central factors are: 1) access to cats with DJD, and 2) outcome measures (both subjective and objective) that distinguish between cats with and without DJD and that are responsive to therapeutic interventions.

In this dissertation, we will describe studies designed to address the shortcomings in our ability to fully utilize the feline model, specifically *understanding recruitment into clinical trials* (Chapter 2), quantification the *caregiver placebo effect* and strategies to mitigate this effect (Chapter 3), and *refinement of subjective and objective outcome measures* (Chapters 3 and 4). A novel method for analysis of activity monitor data will be presented, as well as discovery phase work in biomarker development (Chapter 4). The final chapter will summarize the key findings from these studies, and discuss areas of future research.

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Chapter 2 : Factors influencing participation in clinical trials

Introduction to Chapter 2

As stated in Chapter 1, clinical research studies in cats offer a unique opportunity to study naturally occurring DJD with cats in their home environment. These studies may take the form of research into prevalence, risk factors for development of disease, and efficacy of treatments to address pain or stop the progression of disease. They represent a significant commitment of time and effort from owners and veterinarians. The importance of the ability to recruit representative cases and generalize findings cannot be understated. Given the time and expense of these studies, it is surprising that there is very little guidance or research into the conduct of clinical trial recruitment in veterinary medicine. Our clinical research program has found that recruitment of cats is often difficult, and owner willingness to participate is influenced by study design, including number of visits needed. Knowledge about the features of studies that make owners more or less willing to participate would allow for study design and educational materials to be targeted for successful enrollment of cats. This led us to conduct a survey of cat owners to determine the factors that could influence their participation in clinical trials, presented as part A of this chapter. One clearly influential factor that arose from this survey was recommendation of the clinical trial by their veterinarian. Though physician participation in or recommendation of clinical trials is a well-researched area of human medicine, no studies have been published investigating veterinarian perceptions of clinical trials. This is important to understand, as there is an emphasis in veterinary medicine to practice evidence based medicine, which depends on the successful implementation of clinical trials. In addition, the One Health approach is

increasing awareness of the utility of naturally-occurring models for translational research, and opening the door for parallel drug development and testing. To support growth, we must show that veterinary clinical trials can be recruited for and completed efficiently, making it important to understand the features that contribute to recruitment success or failure. Part B of this chapter reports on the conduct of two cross-sectional survey studies we performed of veterinarians to address this.

Chapter 2a. Clinical trials involving cats: What factors affect owner participation?

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Clinical trials involving cats: What factors affect owner participation?

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Abstract

Clinical trials are frequently hindered by difficulty recruiting eligible participants, increasing the timeline and limiting generalizability of results. In veterinary medicine, where proxy enrollment is required, no studies have detailed what factors influence owner participation in studies involving cats. We aimed to investigate these factors through a survey of owners at first opinion practices.

The survey was designed using feedback from a pilot study and input from clinical researchers. Owners were asked demographic questions and whether they would, would not, or were unsure about participating in a clinical trial with their cat. They then ranked the importance and influence of various factors on participation using a 5-point Likert-type scale, and incentives from most to least encouraging.

A total of 413 surveys were distributed to cat owners at four hospitals, two feline-only and two multi-species; 88.6% were completed. Data for importance and influence factors as well as incentive rankings were analyzed overall, by hospital type, location and whether owners would consider participating.

The most influential factors were trust in the organization, benefit to the cat and veterinarian recommendation. Importance and influence factors varied by willingness to participate. Ranked incentives were not significantly different across groups, with “Free Services” ranked highest.

This study provides a first look at what factors influence participation in clinical trials with cats. Given the importance placed in the recommendation of veterinarians, continued work is needed to determine veterinarian related factors affecting clinical trial participation. The results provide guidance towards improved clinical trial design, promotion and education.

Keywords

Clinical trial, research, cats, participation, proxy

Introduction

Critical to the success of a clinical trial in both human and veterinary medicine is the timely recruitment of a representative sample of participants. Even the best designed and well-funded studies can be hindered by a lack of enrollment. Sluggish enrollment can result in a lengthened trial, which delays publication of results, and poor target population recruitment might limit the generalizability of the findings.¹ The number of participants is further decreased in some pediatric or geriatric studies when the decision for enrollment lies with a proxy and caregiver/parental permission for entry must be sought.² In addition, some populations, such as rural or minority subject, might be underrepresented in clinical trials³

Several studies⁴⁻⁶ have been conducted examining the factors that affect a person's decision to enter a clinical trial, either as a patient or as a caregiver granting proxy consent for entry. A meta-analysis of 33 cancer studies reviewed the barriers to participation for enrolled or eligible clinical trial participants and found that the most frequently reported barriers were related to the protocol design and to patient or physician factors.⁴ Common barriers cited included dislike of randomization or the presence of a placebo group, transportation problems, fear or mistrust of research or researchers, and primary physicians' attitudes toward the trial.

In a review of proxy consent in pediatric research, reasons for granting consent included concerns about a child's illness and the desire to learn more about the medical treatment as well as such altruistic reasons as benefitting others and contributing to medical research.⁵ The same review found that reasons for declining consent included fear of side

effects and inconvenience associated with the protocol. Many of the published studies evaluate patients or their proxies who have enrolled in a clinical trial, rather than a sampling of potential enrollees. A study that investigated parents shortly after they gave, or declined, entry into a clinical trial in a neonatal unit found that perceived risk or benefit of the treatment correlated most strongly with declined or granted authorization respectively.⁶

Clinical trials in companion animal veterinary medicine, by necessity, rely on proxy enrollment. Informed consent is presented to owners, and owners grant permission for their pet to participate. These clinical trials are critical to the field and have advanced therapies in medicine,⁷ pain management,⁸ cardiology,⁹ and behavior¹⁰ among others. Controlled trials have also critically evaluated therapies already in clinical use,¹¹ helping to better inform clinical practice. In addition, species differences in metabolism and tolerance for various drugs¹² make it particularly important to evaluate safety and efficacy of putative treatments in the target species and target population through clinical trials. As in human medicine, clinical trials in companion animal veterinary medicine, particularly in cats, are hampered by difficulties with timely recruitment of eligible animals (unpublished data). One obvious reason for this would be the current low percentage of cat-owning U.S. households that regularly take their cats to veterinary hospitals.¹³ However, by querying cat owners who were bringing their pets to veterinary hospitals (potential enrollees), we aimed to determine the factors influencing clinical trial participation among owners actively seeking veterinary care. To our knowledge, no previous study has investigated the reasons that such people would, or would not, participate in a clinical trial with their pets.

The objectives of the current study were to investigate the factors that are important to people when considering participation in a clinical trial with their cat, detail those factors that make it more or less likely that they would participate, and determine whether demographic features such as location or hospital type (feline-only vs. multi-species) influenced these factors. Additionally, we aimed to evaluate factors that were important and influential to people who indicated interest in participation versus those who are unsure about participation. Finally, the study investigated what incentives would encourage people to participate in a clinical trial with their cat.

Methods

Development of Survey

The survey was constructed with input from experts in feline clinical research, as well as from cat owners. The survey collected limited, relevant demographic input, information about the factors important to cat owners when considering participation in a clinical trial and factors that would encourage or dissuade an owner's participation in a clinical trial with their cat. Factors of importance (termed the importance scale) affecting the owner's decision to participate were rated on a Likert-type scale from 1 to 5, with 1 being "not important at all" and 5 being "very important". The next section concerned factors of influence (termed the influence scale), rated from "much less likely" to "much more likely" for how they would influence an owner's participation. The final section asked participants to rank a list of incentives from the most encouraging, scored at "1" to the least encouraging, scored at "5". Owners were also able to identify and rank an "other" incentive that would be encouraging for participation. At the end of the survey participants could share additional comments they

had on clinical research studies or about the survey. A pilot study was conducted; 15 cat owners visiting North Carolina State University Veterinary Health Complex and 25 cat owners visiting a local veterinary hospital were included and gave feedback on the survey. Feedback on items such as clarity of the questions, time spent completing the survey and feasibility of the survey was used in the development of our final version.

Cat Owner Selection and Practices

Selection of the practices was done in early June 2013 with initial practice meetings taking place in June and July. In-house meetings consisted of the investigators meeting with an individual, identified by the practice and designated a survey leader, responsible for coordinating the distribution of the survey. These individuals formed the communication channel with investigators and were contacted by investigators every 2-3 weeks for updates on progress, goals and needs. Survey leaders held roles such as veterinarian, office manager or technician at the practice. To be eligible to complete a survey, pet owners were required to have at least one cat. Only one survey could be completed per household. Of the 4 practices chosen to participate 2 were feline-only and 2 were multi-species. Two practices, one feline-exclusive and one multi-species were in Location A, and the remaining two were in Location B, both cities within the Triangle Region of North Carolina.

Data Collection

Surveys were administered at a time chosen within the client's visit by each individual practice. If an owner declined, the survey was taken back and marked as a declined survey with the reason being noted, if given. All responses were kept anonymous.

The survey was administered over a two month period, and based on anticipated appointments; a goal was set for the multi-species hospitals to complete 100 surveys and the feline-exclusive hospitals to complete 150 surveys. A free breakfast for the practice was offered as incentive for reaching these goals. Completed surveys were collected by the investigators throughout the administration period. The number of surveys completed and progress towards the goal was discussed with the practices throughout the administration time period.

Statistical analysis

Statistics were performed using a statistical software package (JMP 9.0, Cary NC) with the critical value for significance set at $p \leq 0.05$. Hospitals were identified individually, by type (cat only vs. multi-species) and location (Location A vs. Location B). Descriptive statistics were calculated to describe survey completion, prior participation, whether caregivers would consider participation, number of cats and dogs in the home, whether caregivers could medicate their cats, and preferred method of administering medication. Completion rate over the two months was calculated both as number of completed surveys divided by number of total surveys returned and as the number of completed surveys divided by 150 for feline-only hospitals and 100 for multi-species hospitals (the largest number of potential surveys an individual hospital or each type was expected to complete). Proportions were analyzed using non-parametric tests as indicated; Spearman's rho values were calculated for correlations between factors.

Data from the importance scale were entered as interval level data from 1-5, where 1=least important and 5=most important for analysis. Means were calculated to rank importance factors, and Chi-square or Fisher's exact tests were used to evaluate differences in the distributions of responses between respondents that would participate (Would group) and those that were unsure about participating in a clinical trial with their cat (Unsure group). Data from the influence scale were converted to interval ranges from 1-5 where 1=much less likely, 3= neither more or less likely, and 5=much more likely. Means were calculated to rank importance factors, Wilcoxon rank sum tests were used to evaluate whether influence scale factors differed from the null value of 3, and Chi-square or Fisher's exact tests were used to evaluate differences in the distributions of responses between respondents the Would and the Unsure groups.

Results from the ranking of incentives revealed several issues. Many respondents failed to rank all options, or applied the same rank to multiple options. Therefore, for further analysis, incentives were coded as follows: first, a category was created for each incentive that indicated whether that option had been included in a respondent's top three. A "yes" response could be achieved by assigning a 1, 2, or 3 to the item, having checked the item alone or with 1-2 other items, or by assigning a 1 or 2 or 3 to 1-3 items. If a respondent checked more than three items or assigned ranking to items such that it was impossible to determine the top 3 (without order), the data from that question were discarded. Second, a new category was created called "Which #1" whereby if a respondent had assigned a 1 or placed a check mark by a *single* item, that item was considered their top choice. Data from

respondents who did not assign a 1 or place a check mark by a single item were discarded from this category.

Results

A total of 413 surveys were returned, with 366 (88.6%) completed. In general, a reason for the non-completion was not noted on the survey. Completion rate (the number completed over the number returned) over the study period varied by hospital and ranged from 75.5% for one multi-species hospital to over 90% for the remaining hospitals. Hospital completion (returned over goal) also varied by hospital from 46% for one hospital to 116% for another.

Overall, cat caregivers had a mean of 2.15 cats in the home (range 1-11), this did not vary by hospital type (t-test, $p=0.09$) but did vary by location (t-test, $p=0.0004$) with owners from Location A owning significantly fewer cats than owners at Location B (Table 1). Number of dogs in the home ranged from 0-10, with a mean of 0.39 and no significant difference by hospital location (t-test, $p=0.11$) There was a significant difference by hospital type (t-test, $p<0.0001$) with respondents from feline-only hospitals owning significantly fewer dogs than respondents from multi-species hospitals. However, there was a significant difference, by hospital type, in the number of people that owned no dogs (Chi-square, $p<0.0001$) with 87% of the respondents from the feline-only hospitals owning no dogs, while only 56% of respondents at the multi-species hospitals owned no dogs. The odds ratio for owning more than zero dogs if being a respondent at feline only hospitals vs. multi-species hospitals was $OR=0.188$ (95% $CI=0.114, 0.309$).

Table 1. Mean (\pm standard error) number of cats and dogs owned by respondents at each hospital type and location.

	Hospital Type		Hospital Location	
	Feline-only	Multi-species	Location A	Location B
Cats	2.16 (\pm 0.14)	2.14 (\pm 0.10)	1.94 (\pm 0.10)	2.54 (\pm 0.13)
Dogs	0.21 (\pm 0.05)	0.75 (\pm 0.07)	0.49 (\pm 0.08)	0.33 (\pm 0.06)

Owner responses on the ability to medicate their cat did not vary by hospital type (Fisher's exact, $p=0.1711$) but did vary by location with respondents from Location A indicating that they were less able to medicate their cat (Fisher's exact, $p=0.0009$). For preferred form of medication for their cat, there was a fairly even distribution across the different methods, with 26% of respondents indicating pills as their preferred form, 26% indicating flavored liquid, and 33% indicating flavored chew. The remaining respondents indicated a combination of forms with 7% preferring either a pill or flavored chew. Preferred form did not vary by hospital type (Chi-square, $p=0.41$) or location (ChiSquare, $p=0.69$).

Clinical trial participation

In general, prior participation in a clinical trial was low, with 95% of respondents indicating that they had not previously participated in a clinical trial with their cat. However, this varied significantly by hospital (Fisher's exact, $p=0.0013$) with 12% of respondents from one cat hospital having participated in a clinical trial with their cat in the past. Of the respondents who had prior participation in a clinical trial, 71.4% indicated that they would consider participating in a clinical trial with their cat and 28.6% indicated that they were unsure about participating; no one in this group indicated that they would not participate in a clinical trial with their cat.

Overall, 38% of respondents indicated that they would consider participating in a clinical trial with their cat, 44% were unsure about participation, and 18% indicated that they would not consider participating. The distribution of cat caregivers who would consider participating did not vary by hospital (Fisher's exact, $p=0.158$) or hospital type (Fisher's exact, $p=1.00$), but did vary by location, with respondents from Location A being less likely to consider participating, regardless of hospital type (Fisher's exact, $p=0.0412$). Number of cats in the home also significantly influenced participation, such that respondents who would consider participating or were unsure about participating had more cats in their home than respondents who would not consider participating (ANOVA, $F=6.3726$, $p=0.0019$).

Importance scale

Overall results for each factor are illustrated in Table 2, which shows the factors in decreasing order of importance as well as the proportion of respondents indicating each answer. Several of these factors were found to vary significantly by whether respondents would, would not, or were unsure about participating in a clinical trial. The proportion of respondents in the "Would" and "Unsure" groups who assigned a high importance score (4 or 5) to these factors are detailed in Table 3. One importance factor, "Benefit to my cat," varied by hospital type (Chi-square, $p=0.0294$) with 92% of respondents from cat hospitals and 86% of respondents from multi-species hospitals assigning an importance score of 4 or 5 (high importance). In addition, the factor "My veterinarian recommended the trial" varied by location (Chi-square, $p=0.0185$) with 75% of respondents from Location A and 89% of respondents from Location B assigning an importance score of 4 or 5. In general, importance scores for each factor were lower in the group of respondents who indicated that they would

not consider participating in a clinical trial with their cat, except for the factors “Friend recommended the trial” and “Benefit to my cat”. Correlations between the importance factors were analyzed, and the most highly correlated factors were “Benefit to other cats” and “Advancement of veterinary medical knowledge” ($r=0.5934$, $p<0.0001$), and “Number of trips/visits my cat would have to make for the trial” and “Number of trips/visits I would have to make for the trial” ($r=0.7602$, $p<0.0001$).

Table 2. Importance scale scores. Factors are listed in decreasing order of importance (top down). The mean importance score and proportion of respondents indicating each response is shown.

	Factors (overall mean importance score)	1 Not at all important	2	3	4	5 Very important
Decreasing order of importance 	Trust in the organization performing the trial ^b (3.61)	0.02	0.01	0.06	0.14	0.76
	Benefit to my cat ^c (3.58)	0.02	0.02	0.06	0.18	0.73
	My veterinarian recommended the trial ^{bd} (3.21)	0.06	0.03	0.12	0.24	0.56
	Number of trips/visits my cat would have to make for the trial ^{ab} (3.14)	0.04	0.04	0.15	0.30	0.48
	Benefit to other cats ^{ab} (3.11)	0.02	0.03	0.18	0.35	0.42
	Ability to withdraw from the trial at any time ^b (3.09)	0.03	0.05	0.18	0.29	0.45
	Number of trips/visits I would have to make for the trial ^{ab} (2.84)	0.06	0.08	0.22	0.28	0.37
	Advancement of veterinary medical knowledge ^{ab} (2.75)	0.03	0.07	0.27	0.36	0.26
	If a friend of mine recommended the trial (1.36)	0.29	0.26	0.29	0.12	0.04
Overall proportion of respondents that indicated each level of importance						

^a = Factor for which a significant difference was found between respondents that would or were unsure about participating in a clinical trial with their cat (see Table 3 for analysis).

^b = Factor for which a significant difference was found between respondents that would not participate in a clinical trial, and those that would or were unsure.

^c = Factor that varied significantly by hospital type.

^d = Factor that varied significantly by hospital location.

Table 3. Proportion of respondents indicating a 4 or 5 on the importance scale by whether they would (Would) or were unsure about participating in a clinical trial with their cat (Unsure) for those factors with a significant difference between these two categories.

Factor	Would	Unsure
Number of trips/visits my cat would have to make for the trial Fisher's exact test, $p < 0.0001$	0.85	0.71
Benefit to other cats Fisher's exact test, $p = 0.022$	0.74	0.84
Number of trips/visits I would have to make for the trial Fisher's exact test, $p = 0.009$	0.70	0.60
Advancement of veterinary medical knowledge Fisher's exact test, $p < 0.0001$	0.50	0.82

Influence scale

Overall results for each factor are illustrated in Figure 1, which shows the factors in decreasing order from much more likely to much less likely. All factors except “Approved in the US to treat other conditions in cats” were ranked as significantly different from the null value of “Neither more or less likely” ($p < 0.004$ for each). All factors on the influence scale were found to vary significantly by whether respondents would, would not, or were unsure about participating in a clinical trial with their cat. Proportion of respondents in the Would and Unsure groups who responded that a factor on the influence scales would make them “Much less likely” or “Less likely” (for factors with a mean influence score less than 3) or “Much more likely” or “More likely” (for factors with a mean influence score more than 3) are detailed in Table 4. No influence factors varied significantly by hospital type or location.

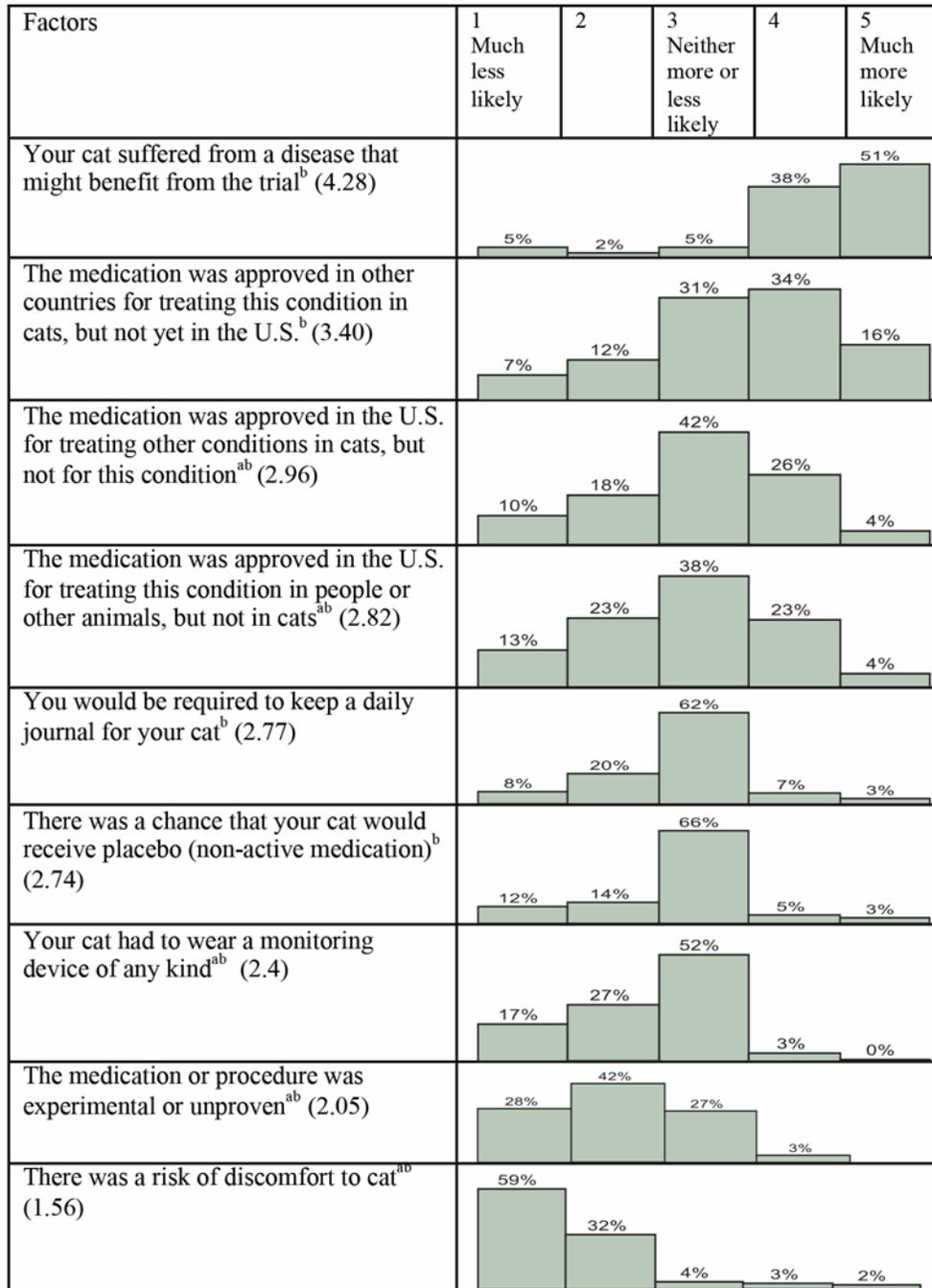


Figure 1. Distribution of Influence scale scores. Factors are listed in decreasing order of importance (top down). The mean influence score and percentage of respondents indicating each response is shown.

Table 4. Proportion of respondents in the Would and Unsure groups that responded that a factor on the influence scales would make them ‘Much less likely’ or ‘Less likely’ (for factors with a mean influence score less than 3) or ‘Much more likely’ or ‘More likely’ (for factors with a mean influence score more than 3) to participate in a clinical trial. P-values from Fisher’s exact tests are provided for factors with a significant difference in distribution between the two groups.

<i>Much less likely and less likely</i>		
	Would	Unsure
Risk of discomfort to cat, p<0.0001	0.89	0.95
Experimental medicine or procedure, p=0.011	0.60	0.76
Cat would have to wear monitoring device, p=0.002	0.30	0.50
Cat might get placebo	0.21	0.25
Daily journaling	0.21	0.29
Approved for this condition in people but not in cats, p=0.015	0.29	0.39
Approved in the US to treat other condition in cats, p=0.001	0.17	0.32
<i>Much more likely and more likely</i>		
	Would	Unsure
Approved for cats in other country	0.57	0.50
Cat has the disease the trial is designed for	0.93	0.89

Incentives scale

Data from the incentives scale were available for analyzing “Which #1” for 311 surveys where a single incentive was ranked highest (85.0% of completed surveys). The incentive ranked as most encouraging overall was “Free Services (bloodwork, exams, etc.),” being given a ranking of #1 on 228 of 311 surveys (73.3%) where a single incentive was ranked highest. Conversion to Y/N for whether an incentive was in a respondent’s top three allowed data to be analyzed for 356 surveys (97.3% of completed surveys). The incentives most likely to be listed in the top three included “Free Services,” “Financial Incentives,” and “Gifts for my cat” (Chi-square, p=0.0001 for each). Distribution of incentives rankings did not vary by hospital type or location.

Twenty-two respondents ranked the “Other” option as most encouraging, 19 of these respondents left a comment. These comments were able to be grouped into categories: benefit to the respondent’s cat (12), benefit to other cats (5), interest in transparency of trial results (1) and a donation to an animal shelter (1).

Discussion

This study offers the first look at the factors that affect an owner’s participation in a clinical trial with their cat. Our findings parallel results from studies of human clinical trial participation, particularly where proxy consent is needed, as in pediatric clinical trials.

Trust in the organization performing the trial and recommendation of the veterinarian were ranked among the most important factors in considering participation in a clinical trial. This finding is similar to that in human studies, where physician recommendations and trust in the organization are frequently cited as motivating factors for clinical trial participation,^{5,14-16} and is an important consideration when designing and recruiting for clinical trials in veterinary medicine. In a study of pediatricians’ attitudes toward clinical trials, pediatricians acknowledged their role in supporting enrollment among their patients.¹⁷ The study found that some pediatricians viewed randomized clinical trials as a hindrance to the individual doctor-patient relationship and voiced concerns regarding lack of control over interventions used during a trial. Veterinarian-client-patient relationships are similarly strong, and veterinary input was rated as very important by 73% of respondents. Support from veterinarians for clinical trials and recruitment through veterinary hospitals might be the most effective way of enrolling the most willing participants.

Over a third of respondents across the hospitals included in this study were unsure about participation. For those in this “Unsure” group, several factors in both the importance and influence scales were found to be different from those respondents who would consider participating in a clinical trial with their cat. This is an outcome that should be of particular importance to clinical researchers, as respondents in the unsure group represent an important source of potential cases. The number of trips with and without their cat were rated as less important factors for considering participation in a clinical trial with their cat for members of the “Unsure” group vs. the “Would” group, while benefit to other cats and advancement of veterinary medical knowledge were rated as relatively more important. Altruistic motivations have also been noted in studies of human clinical trial participation.^{16,18} The ability to withdraw at any time was rated as moderately to very important for 74% of respondents, with no difference between the “Unsure” and “Would” groups. This was also found to be a salient feature for parents in a study of parental awareness of informed consent, where 91% of parents remembered being told this prior to enrolling their child in a clinical trial.¹⁹ When designing a clinical trial, as well any educational or promotional items related to the trial, focusing on factors that are important or influential to this unsure group could enhance participation and enrollment.

Respondents who indicated they would not consider participating in a clinical trial with their cat were different from those who would or who were unsure for many factors. The design of the survey made it impossible to evaluate their reasons for not considering participation, or whether there were any situations in which they might consider participation. The only factor ranked as highly important where this group was not

significantly different from the “Would” and “Unsure” groups was for the importance rating of “benefit to my cat,” suggesting that this factor is important to respondents in this group. However the survey design and data collected did not allow investigation of whether any situation might arise in which they would consider participation. It is also possible that respondents were considering the particular cat that they currently owned, and their responses could reflect individual reasons unique to a specific individual animal, rather than whether they would ever consider participating. This may also have affected the finding that owners with more than one cat were more likely to consider participation, as these owners might have been more likely to have what they would consider a ‘suitable clinical trial participant’ in their home at the time of the survey. This is a bias that may have influenced all of the data, however the data are still relevant as clinical study recruitment will depend on owners enrolling the cat they currently own. A final possibility is that these respondents did not feel sufficiently knowledgeable about clinical trials to respond. In order to avoid a lack of awareness or understanding about clinical trials in general, a small introductory paragraph was included with our survey, but a further question about awareness of clinical trials with cats would have been valuable. No respondent indicated they did not know such trials existed, but we also do not know if lack of knowledge played a role for those who chose not to complete the survey at all.

The findings from the influence scale revealed several interesting results. Risk of discomfort to their cat was the factor that most strongly influenced people against participation, with 59% of respondents indicating that this would make them much less likely to participate. This is similar to a recent study of parental factors involved in pediatric

diabetes trials in which parents were less comfortable with trials involving potentially painful procedures and rated risk of side effects as most influential in determining participation in a trial.²⁰ In the current study, if a trial involved an experimental medicine or procedure, a majority of owners responded that they would be less (42%) or much less (28%) likely to consider participating. This is important as development of new treatments will necessarily go through a stage of being “experimental,” though safety data might be provided. These two factors, along with the need for cats to wear a monitoring device of some type, were significantly different between those in the “Would” and “Unsure” groups, with those in the “Unsure” group providing more extreme responses (toward the end of the scale) than those in the “Would” group. Reasons for this are unknown, but further work that includes questions about owners’ personal views of research, would provide further insight into the differences between these groups. Again understanding the motivations of this group is important if potential recruitment to clinical trials is to be optimized.

Several factors, despite having mean influence scores that were significantly different from the null, had a majority of responses (>50%) indicating that the factor would not make the respondent more or less likely to consider participation. The factors “Your cat had to wear a monitoring device,” “There was a chance that your cat would receive placebo,” and “You would be required to keep a daily journal” appear less influential for cat owners considering participation in a clinical trial with their cat, though the monitoring device was more influential for those in the “Unsure group,” where 50% said that this factor would make them less or much less likely to consider participating. This is in contrast to findings from human studies, where the inclusion of a placebo group is often considered a barrier to

parental consent for a clinical trial.^{14,21} One study on humans found that comfort with placebos was strongly positively correlated with willingness to enroll a child with diabetes into a clinical trial.²⁰ For those factors where respondents indicated that it would make them more or much more likely to consider participating (“Approved for cats in another country” and “My cat has the disease the trial is designed for”) the “Would” and “Unsure” groups were similar in their distribution of responses.

Concerns about transportation required for participation in a clinical trial were not directly queried, though the survey included items about number of trips with and without the cat as part of the importance scale. In the free text area, several respondents commented about time constraints, or the stress of travel with their cat, so inquiring only about the number of trips with or without their cat might not have entirely encompassed concerns regarding travel among the respondents. Transportation issues are a potential hindrance to participation in human clinical trials,²⁰ particularly for rural populations,⁴ and our inclusion of clients at moderately urban hospitals in this study could have decreased this concern among respondents. However, we consider the stress of travel with the cat to be of particular concern to cat owners. One respondent commented that their cat cannot give permission to be part of a clinical trial, so they would not consider participation. The inability for the patient to give permission is often cited as a barrier to participation in pediatric human trials^{14,20,21} and Alzheimer’s research,¹⁶ and is a reasonable concern in veterinary medicine, however it was only mentioned once. The difference between our results and those in human medicine might be that caregivers of pediatric and Alzheimer’s patients could envision a time when the individuals involved would be, or would have been, able to give informed consent.

This study did have some important limitations. While the number of respondents to the survey was reasonable, the small sample size for hospital type (cat versus mixed) and location (A versus B cities) limits the extension of the findings based on these two categories. While our results did reveal some significant differences by hospital location, the two locations are both moderately urban, though Location A had a population of 239,358 and a median income of \$46,136 in 2011, while Location B had a population of 20,591 and median income of \$74,323 in the same year.²² In addition, while the survey did not ask about clinical trials that would be conducted at the NCSU College of Veterinary Medicine, owners might have inferred this location when answering questions about whether they would consider participating, and this could reflect their personal experiences or feelings about the College, or the relative distance from the College (25 miles for Location A vs. 9 miles for Location B).

Volunteer bias is always a concern in survey studies.^{23,24} Attempts to limit this included capturing the percentage of people who did not complete the survey. Compliance with completion was generally high (over 75%) however the number of completed surveys varied by hospital type, which might reflect the lower number of cat appointments seen at multi-species hospitals, or a volunteer bias rooted in the hospital's ability or interest in distributing out the survey to cat owners. While the number of people who did not complete the survey when offered was captured, when it was not, the reasons why the survey was not completed were rarely detailed.

Regional differences in views of clinical trials might vary among cat owners. While differences existed between Location A and Location B, both were moderately urban areas, thus limiting the ability to extend these findings to rural cat owners. In fact, for human clinical trials, recruitment efforts are thought to be more effective at the community, rather than the physician, level.²⁵ The area surrounding the four hospitals involved in this study is a research-intensive area, and respondents could be biased by this fact. However, we feel the results are valuable as the hospitals involved in the present study represent our catchment area for clinical research, and are likely similar to other research institutions where clinical trials with cats are performed. A more widely distributed survey, such as one posted on-line, could address issues of regionality, but are subject to greater volunteer biases than the current study.

In conclusion, this study showed that a substantial portion of cat owners who visit animal hospitals either would consider or are unsure about participation in a clinical trial with their cat. Veterinarian recommendation and trust in the organization were ranked as important factors in the decision about participation. Future work to investigate factors that influence veterinarian participation in or recommendation for a clinical trial might shed further light onto the difficulty with recruitment commonly seen. Free services such as labwork are the most encouraging incentive that can be offered by a trial, and though trial features are clearly important, trial design, advertisement, and explanation emphasizing the benefit to cats and encouraging the endorsement of primary care practitioners can enhance participation in clinical trials with cats.

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Chapter 2b. Veterinarians' Attitudes Toward Clinical Research: Two Cross-sectional Surveys

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Abstract

Objective

Enrollment in clinical research trials is hindered by low recruitment, extending the timeline and cost for research and limiting the generalizability of findings. In veterinary medicine, recruitment relates to owners as well as the pets. Previous research showed recommendation from a veterinarian is important to cat owners considering participation in clinical trials. However, veterinarian attitudes toward clinical research, and factors influencing participation and recommendation of clinical trials are unknown. This study sought to survey veterinarians in small animal practice and ascertain their attitudes concerning participation of client-owned animals in clinical research.

Design

Cross-sectional survey-based study.

Sample Population

815 veterinarian respondents across two surveys.

Procedures

Two on-line surveys were developed and made available to small animal practitioners. The surveys included questions designed to explore factors influencing veterinarians' willingness to recommend clinical trials.

Results

Participation in clinical research was low, with over 80% of respondents from each survey not participating in clinical research. Respondents who did participate were more likely to recommend trials for novel therapies and those sponsored by academic institutions. Distance to clinical trial centers, time restrictions, and lack of awareness of available clinical trials were noted as reasons for not recommending a clinical trial, with 30% of respondents indicating they do not usually hear about clinical trials. The majority of respondents rated their recommendation as important to owners considering participation in a clinical study.

Conclusions and Clinical Relevance

Based on the responses received, participation in clinical research is low among veterinarians and awareness of available clinical trials could be increased. The survey identified factors that are important in clinical trial design and could increase participation from veterinarians.

Keywords: Clinical trial, recruitment, survey, veterinarian

Introduction

Clinical trials are a critical step in the development and testing for therapeutics in human medicine. Through clinical trials in human patients, safety and efficacy are determined in the target population. However, recruitment for clinical trials is often difficult, with low enrollment rates and often disproportionate representation with a bias toward those with the requisite flexibility and transportation to participate. This extends the timeline and increases costs for trials, while also limiting the generalizability of findings.¹ Barriers to participation in clinical trials have been studied in human medicine, and they frequently include a lack of awareness of available clinical trials, concerns about randomization, and logistical issues, such as transportation to clinical trial centers.²⁻⁴ People who do participate in clinical trials often cite either hope for personal benefits, such as relief from a condition, or altruistic reasons including benefit to future patients and advancement of clinical medicine.⁵ A systematic review of methods to improve recruitment and enrollment in clinical trials found that increasing understanding of the condition being studied, rather than understanding of the clinical trial process was able to increase enrollment.⁶ Physicians, emphasizing their roles in clinical trial participation, provide much of this increased understanding.

Clinical trials in veterinary medicine are equally important, as safety and efficacy cannot be reliably extrapolated from one species to the next. The presence of a profound caregiver placebo effect⁷⁻¹⁰ further highlights the need for optimal recruitment and enrollment to produce appropriately powered clinical trials with representative populations. In veterinary medicine, it is necessary to recruit both the owner and the pet, thus introducing

a dynamic based on a triad of care, and highlighting the importance of the veterinarian-client-patient relationship.

This dynamic is also present in human medicine with clinical trials involving patient groups unable to provide informed consent, particularly pediatric populations. Much has been written about the issues surrounding recruitment and consent in these populations.^{11,12} Personal relationships with physicians and trust in their recommendations are often integral to the decision to participate in a clinical trial.^{11,12} In clinical trials with pediatric patients, it has been shown that impersonal recruitment strategies, such as mailings and advertisements, yield fewer participants than recruitment through physicians integrally involved in the cases.¹³ In summarizing two studies of asthma self-management, Knox and Burkhart reported that 76% and 87% of the participants were referred by their physician, despite the presence of child-friendly brochures, flyers, and posters in pediatric settings. The importance of physician recommendation is integral to successful recruitment. However, doctors' reluctance to enroll patients has been called one of the most serious obstacles to trial success, as patients will rarely participate unless actively encouraged to do so by their physician.^{6,14} Further, pediatricians' perceptions of the burden of the trial on patients influences patient refusal rates, suggesting that subtleties in discussion of the trial with caregivers is very influential in a participant's decision to enroll.¹⁵

A previous study of cat owners investigated features contributing to owner participation in clinical trials with their cats.¹⁶ This study identified several features including trust in the organization performing the trial, whether the cat had a disease that would benefit

from the trial, and the recommendation of their veterinarian. In that study, 80% of respondents said that recommendation from their veterinarian was important (24%) or extremely important (56%) in their consideration of participation. While several studies have investigated the features that influence physician participation in and recommendation of clinical trials in human medicine,^{4,15,17-19} this work has not yet been thoroughly performed in veterinary medicine. In human medicine, study design and protocol features have been found to influence physicians' recommendations. In addition, features about the patient can influence whether a physician even raises the topic of a clinical trial.

The objectives of this study were to survey veterinarians in small animal practice and ascertain their attitudes concerning participation of client-owned animals in clinical research. Survey questions addressed the influence of experimental and other trial features on veterinarian recommendations for clinical trial participation. The survey also asked about participants' beliefs about what was important to their clients in considering a clinical trial, and about the best ways to advertise clinical trials to veterinarians.

Materials and Methods

Survey development

Questions for the survey were generated de novo based on expert input and review of the human literature regarding physician views on clinical research and clinical trial participation. Expert input was solicited in two phases, first by a focus group with General Practice faculty and faculty members engaged in clinical trials, and then via feedback from pilot testing and a group of survey evaluators at the Veterinary Information Network (VIN).

An initial 83-item version of the survey was sent to 40 veterinarians for pilot testing, with 35 completing the survey. Average completion time for the pilot survey was 26.1 minutes, with 10 respondents indicating that the length was satisfactory, while the remaining respondents indicated that the survey was either long but feasible (n = 22), a bit too long (n = 2), or way too long (n = 1). Based on the completed responses, changes were made to delete two items from the survey and to clarify or improve items based on free text responses from pilot survey participants.

The final survey consisted of 81 items broken into eight sections. The first section contained eight items querying demographic information, while the second section included questions about prior clinical trial participation. For questions in the next five sections, respondents were asked to rate either the importance or the influence of a number of features in their willingness to recommend a clinical trial or their opinion on the importance of those features to pet owners. Items in the final section were designed to gauge the respondent's general feelings about clinical trials, best ways to advertise the trial, and best incentives for owner/pet participation. An email invitation to participate was sent to a database of 1657 veterinarians and veterinary health professionals held by the United Kingdom based company Vet Professionals Limited (VP). The survey was open for two months, with emails sent to database members on two occasions. The full survey is available in Appendix 2.

Following the close of the initial VP survey, the survey was adapted for distribution to the Veterinary Information Network (VIN) following input from the VIN Survey representative (MR) in order to increase the response number and geographic distribution of

responses. To increase participation, the survey was shortened to 26 items, while retaining the original language as much as possible. A more dynamic survey was generated so that the participants' response on a particular item could branch to subsequent items. The flow of the survey is detailed in Figure 1. The full survey is available in Appendix 3. The survey was sent to 30,088 VIN members (excluding students, academics and veterinarians in industry) and a reminder email was sent 2 weeks later.

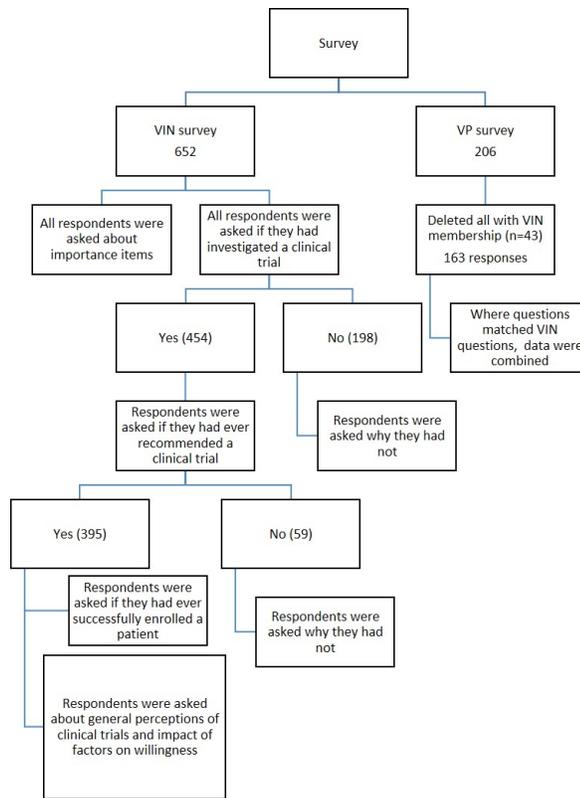


Figure 1. Flow of survey questions and participants

Statistical analyses

Handling of the data

To reduce the possibility of redundancy among respondents, data from respondents in the VP survey who identified themselves as VIN members (n=43) were deleted. In addition, all non-veterinarian responses were deleted. Graduation dates in the first survey were manually coded to comply with the bins used in the VIN survey. These were categorized in bins of 10-year timespans, with the exception of 2010-2014, where only the most recent graduates from the past five years were represented.

For both surveys, Likert scales were converted to numerical rating scales from 1-5. For “importance” items, the scale ranged from 1= irrelevant to 5= essential; “likelihood” items ranged from 1=extremely unlikely to 5=extremely likely with 3=neutral; “agreement” items ranged from 1=strongly disagree to 5=strongly agree with 3=neutral.

Free text, whether part of an “other” option on a multiple-choice question or a free-choice response, was categorized manually by keyword or theme. Responses with an identical or common theme were grouped together.

After conversion to numerical scores, mean values were calculated to rank items for agreement, importance, and likelihood; no statistical testing was carried out using means. Distributions of responses by graduation year category were compared using an analysis of means of proportions. For the VP survey, and for importance items on the VIN survey, distributions of responses were compared between respondents that had, or had not,

recommended a clinical trial in the past using Chi-square or Fisher's Exact tests as appropriate. Given the number of responses to each survey, factor analysis was performed on responses to importance items from the VIN survey only. Factor analysis was used to determine features that were then associated with being more or less likely to investigate or recommend a clinical trial using logistic regression. All analyses were performed using a statistical software program^a, and significance was set at $P < 0.05$.

Results

Following removal of incomplete, non-veterinarian, or VIN member responses, the VP survey resulted in 163 responses with useable data. The VIN survey yielded 652 responses with complete data. Given the dynamic application of the VIN survey, the number of responses for individual questions varied, and the number is indicated in the results where applicable, and detailed in Figure 1.

Demographic information

Results for the distribution of graduation year and type of practice from each of the surveys are shown (Figure 2 A&B). Respondents on the VIN survey mainly practice in the United States ($n=526$, 80.7%), with 81 respondents (12.4%) in Canada, and 45 respondents from other countries (no other country had $>5\%$ of respondents, the next most frequent was Australia with 2.5%). In contrast, respondents on the VP survey mainly practice in the United Kingdom ($n=136$, 83.4%), with 27 respondents (16.6%) from other countries (no country had $>5\%$ of respondents). The majority of respondents from each survey self-identified as being veterinarians whose practices included small animal +/- exotics (77.1%

and 83.4% for VIN and VP surveys, respectively) or mixed animal (5.2% and 4.3% for VIN and VP surveys, respectively).

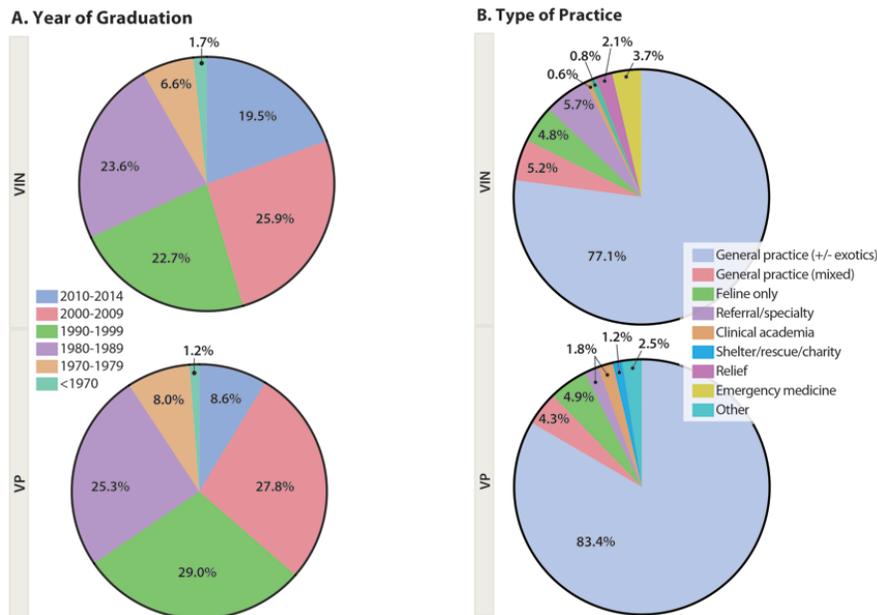


Figure 2. Distribution of year of graduation (A) and type of practice (B) for each survey (VIN and VP).

Time spent in clinical research

The vast majority of respondents from each survey (VIN: 81.7%; VP: 88.3%) spent no time in clinical research, or <10% of their time in clinical research (VIN: 16.4%; VP: 4.9%).

Investigation of clinical trials and barriers to participation

VIN survey responses

Respondents that had never investigated enrolling a patient in a clinical trial (Figure 1) (n=198) were asked their reasons why they had not; 179 provided responses. The reasons selected by the most respondents included “Clinical trial centers are too far away from my practice” (37.4%), “Other” (37.4%), and “There are no clinical trials or diseases for patients that I deal with” (30.1%). Among the “Other” reasons provided as free text responses, the most common themes involved a lack of awareness about clinical trials including what clinical trials were available, the process for enrolling a patient in a clinical trial, and where to look for clinical trials.

Respondents that had investigated a clinical trial in the past (n=454) were queried about their frequency of investigation, whether they had ever recommended enrollment, and whether they had successfully enrolled a patient into a clinical trial. Results are summarized in Figure 3.

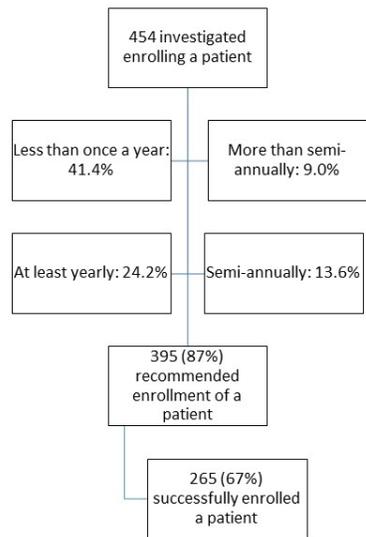


Figure 3. Results from VIN survey for investigation of, recommendation of, and enrollment into a clinical trial by veterinarian respondents.

Respondents who had investigated but not recommended a clinical trial (n=59) cited distance to clinical trial centers (67.8%), availability of clinical trials for their patients (48.2%), and lack of interest from clients (19.6%) as the most common reasons for not recommending enrollment (percentages from 56 respondents that provided reasons). Finally, respondents that had recommended a clinical trial but were unsuccessful in enrolling a patient (n=130) cited distance to clinical trial centers (54.7%), clients not interested (43.6%), and lack of clinical trials available (25.3%) as the most common reasons for failing to enroll a patient (percentages from 126 respondents that provided reasons). Very few respondents indicated that participation in clinical trials was against current practice policy (<1%), or that there was no time for (3.3%) or interest in (8.3%) participating in clinical trials. Less than 1%

of respondents indicated that they did not want to lose the client to the study investigators, while 6.1% indicated that their clients are not interested in participating in clinical trials.

There were significant differences in the distribution of respondents that had investigated or recommended enrollment of a patient by graduation year category (Chi-square, $P < 0.0001$, and $P = 0.0031$, respectively), with fewer than expected of the respondents graduating between 1980 and 1999 investigated enrolling a patient in a clinical trial, while more than expected of the respondents that graduated the most recently (between 2010 and 2014) had investigated or recommended enrolling a patient into a clinical trials. There was no significant difference in the distribution of those who had successfully enrolled a patient by graduation year category (Chi-square, $P = 0.068$), although again it was generally lower for those graduating between 1980 and 1999.

VP survey responses

In the VP survey, participants were asked if they had recommended a clinical trial for a patient in the past, with 44.2% responding with Yes, while 55.8% responded with No. Participants in this survey were not asked about reasons for not recommending a trial. There was no difference in the distribution of graduation year category between those that had or had not recommended a clinical trial in the past (Fisher's Exact, $P = 0.068$).

Agreement items

Responses to agreement items were asked of 395 of the VIN respondents, and all of the VP respondents. For either survey, no significant difference in distribution was found for

any item across the year of graduation groups. The results are summarized in Tables 1 (VIN) and 2 (VP). In general, veterinarians who had recommended a clinical trial strongly agreed that veterinary clinical trials were important in veterinary medicine and that they felt comfortable discussing clinical trials and informed consent. Most respondents from the VIN survey disagreed or strongly disagreed that anecdotal information is sufficient for most therapies (76%), and that their clients were aware of veterinary clinical trials (76%). In the VP survey, no difference in distribution of responses was found for those that had, or had not recommended a clinical trial in the past for the majority of items. However, there was a significant difference in distribution the agreement with the statement, “I feel comfortable discussing clinical trials and informed consent with my clients,” with generally more negative responses from those that had not previously recommended a clinical trial (Chi-square, $P=0.002$) (distribution summarized with other factors in Table 7).

Table 1. Agreement items from the VIN survey. Veterinarians were asked to rate their level of agreement with statements regarding clinical trials in veterinary medicine. Items are listed in order of decreasing agreement as ranked by mean score for agreement.

	Frequency distribution of responses				
	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Veterinary clinical trials are important in advancing veterinary medicine	0.83	0.16	0.01	0.00	0.00
I feel comfortable discussing clinical trials and informed consent with my clients	0.47	0.43	0.08	0.02	0.00
Veterinarians should recommend clinical trials to owners as often as possible	0.19	0.30	0.43	0.69	0.00
Veterinarians should recommend clinical trials ONLY to select clients	0.00	0.34	0.31	0.22	0.12
Most of my clients are fearful about participating in a veterinary clinical trial	0.02	0.15	0.48	0.33	0.02
Most of my clients are aware of veterinary clinical trials	0.02	0.08	0.14	0.56	0.20
Anecdotal information is sufficient for most therapies	0.12	0.04	0.18	0.50	0.26

Table 2. Agreement items from the VP survey. Veterinarians were asked to rate their level of agreement with statements regarding clinical trials in veterinary medicine. Items are listed in order of decreasing agreement as ranked by mean score for agreement. Distributions of responses were compared between those that had and had not recommended a clinical trial in the past using Chi-square or Fisher's exact tests. ^s indicates a significant difference in these distributions (P<0.05).

	Frequency distribution of responses				
	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Veterinary clinical trials are important in advancing veterinary medicine	0.58	0.38	0.04	0.00	0.00
I feel comfortable discussing clinical trials and informed consent with my clients ^s	0.09	0.44	0.31	0.15	0.01
Veterinarians should recommend clinical trials ONLY to select clients	0.00	0.48	0.30	0.11	0.11
Most of my clients are fearful about participating in a veterinary clinical trial	0.03	0.03	0.39	0.27	0.02
Most of my clients are aware of veterinary clinical trials	0.01	0.03	0.07	0.48	0.41

Likelihood items

Three-hundred and ninety-five of the VIN respondents (those who had recommended a clinical trial in the past) and all VP respondents were asked to rate the likelihood that they would recommend a clinical trial to a client based on a set of experimental, methodological, and additional features. Results are summarized in Tables 3 (VIN) and 4 (VP). For the VP survey, there were no differences in the distribution of responses for those that had recommended a clinical trial in the past, and those who had not, with the exception of one item, discussed with the additional factors.

Table 3. Likelihood ratings for trial features from the VIN survey. Veterinarians were asked to rate whether the following features would make them more or less likely to recommend a clinical trial to a client. Items are listed in order of decreasing positive influence as ranked by mean score for likelihood.

<i>Experimental factors</i>	Most Unlikely				Most Likely
	1	2	3	4	5
Trial involves a therapy licensed elsewhere	0.00	0.02	0.09	0.47	0.42
Trial involves a rare disease	0.00	0.07	0.14	0.46	0.33
Trial examines a novel therapy	0.00	0.05	0.19	0.55	0.20
Trial examines an anecdotal therapy	0.02	0.10	0.24	0.48	0.14
Trial involves a surgical intervention	0.01	0.09	0.37	0.44	0.09
Trial examines a new treatment for a disease with a currently successful therapy	0.02	0.23	0.30	0.34	0.11
<i>Methodological factors</i>					
	Most Unlikely				Most Likely
	1	2	3	4	5
Trial is non-invasive	0.00	0.00	0.02	0.36	0.61
Trial is minimally invasive	0.00	0.01	0.05	0.59	0.35
Data collection interferes minimally with daily activities	0.00	0.03	0.10	0.55	0.32
Trial involves invasive sampling procedures	0.02	0.21	0.36	0.36	0.05
Data collection interferes moderately with daily activities	0.02	0.25	0.38	0.32	0.03
<i>Additional factors</i>					
	Most Unlikely				Most Likely
	1	2	3	4	5
Trial is run by a respected investigator	0.00	0.00	0.03	0.47	0.49
You would be informed of the trial results	0.00	0.00	0.06	0.48	0.46
Trial is sponsored by an academic institution	0.00	0.00	0.13	0.46	0.40
Client was compensated for their time	0.01	0.00	0.24	0.50	0.25
You retain management of the case	0.00	0.02	0.31	0.39	0.28
Trial results are guaranteed to be published	0.00	0.01	0.33	0.39	0.26
You were compensated for your time	0.00	0.01	0.46	0.34	0.18
Trial is funded by a corporation	0.00	0.08	0.37	0.42	0.13
Trial is not funded	0.09	0.35	0.40	0.13	0.03

Table 4. Likelihood ratings for trial features from the VP survey. Veterinarians were asked to rate whether the following features would make them more or less likely to recommend a clinical trial to a client. Items are listed in order of decreasing positive influence as ranked by mean score for likelihood. Distributions of responses were compared between those that had and had not recommended a clinical trial in the past using Chi-square or Fisher’s exact tests. [§] indicates a significant difference in these distributions (P<0.05).

<i>Experimental factors</i>	Most Unlikely				Most Likely
	1	2	3	4	5
Trial involves a therapy licensed elsewhere	0.01	0.02	0.30	0.49	0.18
Trial examines a new treatment for a disease with a currently successful therapy	0.02	0.33	0.39	0.19	0.06
Trial involves a rare disease	0.06	0.42	0.34	0.14	0.04
<i>Methodological factors</i>					
	Most Unlikely				Most Likely
	1	2	3	4	5
Trial is non-invasive	0.00	0.01	0.11	0.50	0.38
Trial is minimally invasive	0.00	0.04	0.26	0.55	0.14
Trial involves invasive sampling procedures	0.19	0.53	0.19	0.08	0.00
<i>Additional factors</i>					
	Most Unlikely				Most Likely
	1	2	3	4	5
Trial results are guaranteed to be published	0.00	0.01	0.09	0.47	0.43
You would be informed of the trial results	0.00	0.01	0.10	0.60	0.28
Trial is run by a respected investigator [§]	0.00	0.01	0.16	0.59	0.24
Trial is sponsored by an academic institution	0.00	0.03	0.16	0.60	0.21
Client was compensated for their time	0.01	0.02	0.19	0.62	0.16
You were compensated for your time	0.00	0.03	0.38	0.43	0.15
Trial is funded by a corporation	0.03	0.19	0.64	0.13	0.01

For the experimental features, responses on the VP survey were in the same direction as the VIN survey except for one item, “Trial involves a rare disease” where responses from the VP survey were generally more negative. Among the most positive influential features was that a trial was for a therapy licensed elsewhere, however all features had a positive influence in this group of respondents, with the exception of the one item on the VP survey.

For the methodological features, again, all features had an overall positive influence on the group of respondents to the VIN survey, though the most highly positive included that the trial was non-invasive or minimally invasive. More invasive sampling or disruption of daily activity shifted the distribution toward less likely to recommend the trial.

For the additional features, the distributions of responses to all items were in the same direction from neutral for both surveys, except for one, “Trial is funded by a corporation/industry” where the responses from the VP survey were below neutral. Features that were rated most positively by both surveys were that the trial was run by a respected investigator, the clinician would be informed of the trial results, and that the trial was sponsored by an academic institution. In the VP survey, there was a difference in the distribution of responses between those that had, or had not recommended a trial in the past for the item, “Trial is run by a respected investigator” (Fisher’s Exact, $p=0.042$) with generally more negative responses from those that had not recommended a clinical trial in the past (distribution summarized with other items in Table 7).

Importance items

All participants in both surveys were asked to rate the importance of several features, either to themselves in their decision to recommend a clinical trial to a client, or to their clients in considering participation. A total of 765 respondents (618 from VIN and 147 from the VP survey) provided analyzable data for this section; responses for each survey are summarized in Tables 5 (VIN) and 6 (VP). The items that had higher proportions of increased importance ratings to the veterinarian included belief in the value of the study,

concern about putting the research study needs and protocol before the pet’s needs, and inconvenience to the owner. For the respondents’ beliefs about importance to their clients, many items were rated as very important, including that the pet has a disease that could benefit from the trial, safety of the treatment, cost of participation, and distance to the trial center among others. One feature, that the pet would need to be fasted prior to rechecks, was rated as not important to owners, suggesting that clinicians do not believe fasting a pet is difficult for owners to accomplish.

Table 5. Importance ratings for trial features from the VIN survey. Veterinarians were asked to rate how important they considered certain trial features to be when considering recommendation of a clinical trial to a client, and how important they considered certain trial features to be to their clients in considering participation. Items are listed in order of decreasing importance as ranked by mean score for importance. Distributions of responses were compared between those that had and had not recommended a clinical trial in the past using Chi-square or Fisher’s exact tests. [§] indicates a significant difference in these distributions (P<0.05).

<i>Importance to the veterinarian</i>	Lowest importance				Highest importance
	1	2	3	4	5
Belief in the value of the study [§]	0.01	0.02	0.06	0.48	0.43
Concern about putting the research study needs and protocol before the pet’s needs [§]	0.03	0.10	0.14	0.43	0.29
Inconvenience to the owner	0.03	0.10	0.11	0.58	0.18
Concern about my relationship with my client [§]	0.08	0.12	0.20	0.42	0.18
Time required to assess/treat/enroll the pet	0.03	0.15	0.25	0.49	0.08
Loss of trust from client [§]	0.10	0.15	0.19	0.34	0.22
Paperwork that would be required by the trial	0.04	0.18	0.24	0.44	0.10
Time required to explain the trial to the client [§]	0.13	0.29	0.27	0.28	0.04
Loss of case control [§]	0.20	0.30	0.28	0.17	0.05

Table 5 (continued).

<i>Importance to client</i>	Lowest importance					Highest importance				
	1	2	3	4	5					
Pet has a disease that could benefit from the trial	0.00	0.01	0.00	0.27	0.71					
Safety of the treatment	0.00	0.01	0.00	0.29	0.70					
Cost of participation	0.00	0.02	0.03	0.40	0.55					
Distance to the trial center	0.00	0.01	0.06	0.48	0.45					
Free evaluation and treatment	0.00	0.01	0.08	0.48	0.42					
Number of rechecks required	0.00	0.02	0.07	0.53	0.38					
Veterinarian endorsement of the trial	0.00	0.01	0.05	0.59	0.33					
There was a chance of the pet being in the placebo group	0.01	0.03	0.22	0.50	0.24					
Duration of each recheck	0.01	0.09	0.17	0.50	0.23					
The treatment is experimental/novel	0.01	0.07	0.26	0.51	0.15					
They would be required to keep a daily log	0.01	0.16	0.24	0.46	0.13					
The pet would need to wear a monitoring device ^s	0.02	0.16	0.34	0.38	0.09					
The pet would need to be fasted prior to rechecks ^s	0.01	0.36	0.32	0.19	0.04					

Table 6. Importance ratings for trial features from the VP survey. Veterinarians were asked to rate how important they considered certain trial features to be when considering recommendation of a clinical trial to a client, and how important they considered certain trial features to be to their clients in considering participation. Items are listed in order of decreasing importance as ranked by mean score for importance. Distributions of responses were compared between those that had and had not recommended a clinical trial in the past using Chi-square or Fisher's exact tests. ^s indicates a significant difference in these distributions (P<0.05).

<i>Importance to the veterinarian</i>	Lowest importance				Highest importance
	1	2	3	4	5
Belief in the value of the study	0.00	0.01	0.07	0.45	0.47
Concern about putting the research study needs and protocol before the pet's needs ^s	0.02	0.01	0.12	0.32	0.53
Inconvenience to the owner	0.01	0.02	0.14	0.35	0.48
Time required to assess/treat/enroll the pet	0.03	0.03	0.22	0.48	0.23
Concern about my relationship with my client ^s	0.04	0.11	0.31	0.32	0.22
Paperwork that would be required by the trial ^s	0.02	0.05	0.25	0.40	0.27
Time required to explain the trial to the client ^s	0.01	0.14	0.29	0.35	0.20
Loss of trust from client ^s	0.04	0.16	0.31	0.31	0.19
Loss of case control ^s	0.05	0.18	0.37	0.31	0.10
<i>Importance to client</i>					
	Lowest importance				Highest importance
	1	2	3	4	5
Safety of the treatment	0.00	0.03	0.02	0.20	0.75
Pet has a disease that could benefit from the trial	0.00	0.01	0.03	0.29	0.67
Cost of participation	0.01	0.01	0.03	0.26	0.68
Distance to the trial center	0.00	0.04	0.14	0.44	0.38
Number of rechecks required	0.01	0.01	0.14	0.48	0.36
Veterinarian endorsement of the trial ^s	0.01	0.02	0.18	0.53	0.26
Duration of each recheck	0.03	0.14	0.18	0.40	0.24
They would be required to keep a daily log ^s	0.00	0.14	0.25	0.42	0.18
The treatment is experimental/novel	0.01	0.12	0.36	0.27	0.23
The pet would need to wear a monitoring device	0.00	0.18	0.33	0.36	0.12
The pet would need to be fasted prior to rechecks	0.04	0.27	0.29	0.29	0.10

For both surveys, significant differences were found in the rating of importance items between respondents that had, or had not recommended a clinical trial in the past (Tables 4-5). Interestingly, there was remarkable overlap between the two surveys in the items that were differentially distributed between those that had and had not recommended a trial in the past, with differences in distribution for the items: Concern about putting research study needs before pet's needs, concern about my relationship with my client, loss of trust from a client, loss of case control, and time required to explain the trial to the client. Distribution of responses for all items where a difference was found between those that had and had not recommended a clinical trial in the past are summarized in Table 7.

Table 7. Summary of the distribution of responses for all items where a significant difference was found between those that had, or had not recommended a clinical trial in the past.

	<i>Importance items</i>	<i>Had recommended a trial</i>					<i>Had not recommended a trial</i>				
		1	2	3	4	5	1	2	3	4	5
VIN	Belief in the value of the study	0.01	0.02	0.02	0.47	0.48	0.04	0.04	0.13	0.51	0.29
	Concern about putting the research study needs and protocol before the pet's needs	0.03	0.06	0.11	0.43	0.37	0.07	0.14	0.18	0.44	0.16
	Concern about my relationship with my client	0.04	0.10	0.17	0.47	0.22	0.18	0.14	0.24	0.35	0.09
	Loss of trust from client	0.05	0.12	0.15	0.39	0.29	0.33	0.11	0.29	0.20	0.07
	Time required to explain the trial to the client	0.12	0.29	0.26	0.28	0.05	0.27	0.40	0.18	0.13	0.02
	Loss of case control	0.15	0.27	0.32	0.19	0.07	0.40	0.33	0.22	0.02	0.04
	The pet would need to wear a monitoring device	0.02	0.16	0.31	0.41	0.10	0.07	0.18	0.43	0.18	0.13
	The pet would need to be fasted prior to rechecks	0.07	0.39	0.30	0.18	0.06	0.18	0.24	0.31	0.22	0.04
	<i>Agreement items</i>	<i>Had recommended a trial</i>					<i>Had not recommended a trial</i>				
		<i>Disagree</i>		<i>Agree</i>			<i>Disagree</i>		<i>Agree</i>		
VP	I feel comfortable discussing clinical trials and informed consent with my clients	0.00	0.16	0.54	0.21	0.09	0.05	0.36	0.39	0.19	0.01
	<i>Likelihood items</i>	1	2	3	4	5	1	2	3	4	5
	Trial is run by a respected investigator	0.00	0.01	0.09	0.68	0.22	0.00	0.00	0.23	0.52	0.25
	<i>Importance items</i>	1	2	3	4	5	1	2	3	4	5
	Concern about putting the research study needs and protocol before the pet's needs	0.03	0.00	0.15	0.42	0.39	0.01	0.01	0.10	0.23	0.64
	Concern about my relationship with my client	0.08	0.14	0.30	0.38	0.11	0.01	0.09	0.31	0.27	0.32
	Paperwork that would be required by the trial	0.03	0.05	0.38	0.36	0.18	0.01	0.06	0.15	0.43	0.35
	Time required to explain the trial to the client	0.03	0.20	0.35	0.27	0.15	0.00	0.10	0.25	0.42	0.23
	Loss of trust from client	0.08	0.18	0.35	0.32	0.07	0.01	0.14	0.27	0.30	0.28
	Loss of case control	0.06	0.20	0.47	0.21	0.06	0.04	0.16	0.28	0.38	0.14
Veterinarian endorsement of the trial	0.00	0.05	0.11	0.53	0.32	0.01	0.00	0.24	0.54	0.21	
They would be required to keep a daily log	0.00	0.11	0.26	0.53	0.11	0.00	0.16	0.25	0.34	0.25	

Factor analysis of importance items from the VIN survey

Factor analysis across the 22 importance items was performed for responses from the VIN survey where complete responses were provided (n=609). Six factors explained 60.94% of the total variance in responses (Factor loading pattern shown in Table 8).

Table 8. Factor loading pattern for importance items among VIN respondents (n=434). Those factors that are most influential on overall factor score for Factors 2, 3, and 4 (those with factor loadings greater than ± 0.40) are in bold.

Factor Pattern						
	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
Belief in the value of the study	0.314	0.299	0.376	0.231	0.165	0.318
Concern about putting the research study needs and protocol before pet's needs	0.433	0.353	0.275	-0.116	-0.269	0.159
Inconvenience to the owner	0.444	0.070	-0.023	0.044	-0.495	0.183
Concern about my relationship with my client	0.482	0.584	0.192	-0.310	-0.167	-0.077
Time required to assess/treat/enroll the pet	0.480	0.326	-0.435	0.535	0.106	-0.015
Loss of trust from client	0.465	0.603	0.237	-0.306	-0.124	-0.129
Paperwork that would be required by the trial	0.508	0.274	-0.477	0.491	0.110	0.069
Time required to explain the trial to client	0.432	0.443	-0.467	0.224	0.046	-0.154
Loss of case control	0.414	0.528	0.008	-0.263	0.012	-0.228
Pet has a disease that could benefit from the trial	0.263	-0.033	0.552	0.333	0.153	0.172
Safety of the treatment	0.444	-0.116	0.391	0.036	0.230	-0.011
Cost of participation	0.505	-0.314	0.136	0.187	-0.072	-0.447
Distance to trial center	0.512	-0.475	0.140	0.187	-0.400	0.011
Free evaluation and treatment	0.420	-0.278	0.163	0.172	0.100	-0.467
Number of re-checks required	0.623	-0.416	-0.062	0.074	-0.394	0.077
Veterinarian's endorsement	0.307	-0.005	0.432	0.180	0.276	0.363
Pet in placebo group	0.427	-0.184	0.272	-0.104	0.288	-0.340
Duration of each recheck	0.574	-0.355	-0.131	-0.152	-0.282	0.133
Treatment is experimental	0.461	-0.061	0.186	-0.069	0.256	-0.009

Table 8. (continued).

Need to keep a daily log	0.580	-0.295	-0.285	-0.311	0.310	0.115
Need to wear a monitor	0.617	-0.222	-0.308	-0.319	0.202	0.217
Need to fast prior to recheck	0.468	-0.186	-0.370	-0.366	0.268	0.146

A logistic regression model was fit with the probability that a respondent had investigated enrolling a patient in a clinical trial as the response variable (434 veterinarians had investigated enrollment, while 175 had not). Factors 2, 3, and 4 were all significantly associated with recommending enrollment ($P = 0.0017$, $P=0.0001$, and $P=0.0055$, respectively). Increases in Factors 2 and 3 were associated with increased likelihood of investigating enrollment (Odds ratios [95% Wald confidence limits] of 1.34 [1.12, 1.61] and 1.45 [1.20, 1.74], respectively), while Factor 4 was associated with a decreased likelihood of investigating (Odds ratio [95% Wald confidence limits] of 0.775 [0.65, 0.93]).

Clinical trial awareness

VIN survey responses

In the VIN survey, all participants were asked how they obtain information about available clinical trials, and the most effective ways to promote veterinary clinical trials to veterinarians in their area. At least 40% of participants said that they obtain information about clinical trials from email notifications, word of mouth, and promotions on VIN. Nearly 30% responded that they do not usually hear or know of clinical trials in their area. For the most effective ways to deliver information about clinical trials, 70% of participants selected email to the practice, 60% selected a clinical trials website, and 54% selected printed information sent to the practice. Only 43% selected a visit from the study investigator.

VP survey responses

In the VP survey, participants were also asked how they find information about clinical trials in their area, and the top two ways to promote veterinary clinical trials in their area. As in the VIN survey responses, 36% indicated that they do not typically hear of clinical trials in their area, while 37% selected email and 24.8% selected mailed flyers. Forty-six percent of respondents indicated that they obtained information about clinical trials through journals; this option was not provided in the VIN survey. The responses to the most effective way to promote clinical trials were somewhat different from the VIN survey, with 45.4% of respondents selecting email to the practice, and 53.9% selecting a visit from the study investigator. Only 19.8% selected a clinical trials website for their area/region, while 30.0% indicated that continuing education seminars would be effective. Participants in the VP survey were also asked about their overall feelings about clinical trials in veterinary medicine, as well as what incentives they thought would be best for encouraging owner participation in clinical trials. Overall, 75.2% of respondents had positive feelings about clinical trials in veterinary medicine, with 22.6% neutral and only 2.2% negative. The majority of respondents selected free services (such as labwork, radiographs, and examinations) as the best owner incentive for participation in a clinical trial.

Discussion

Our study demonstrates that small-animal general practice veterinarians are interested in clinical trials in veterinary medicine, and that there appear to be several trial features that are important and influential in the decision to recommend a clinical trial to a client. Understanding these features may enhance our ability to recruit patients for clinical trials,

which benefits animal health as well as supporting the claim that parallel development of drugs for companion animal and human use is a worthwhile endeavor. As very little research has been done in this area with veterinarians, comparisons from our study will also be discussed in light of several insights from physicians in human medicine.

In this study, several survey items were restricted to veterinarians who had recommended a clinical trial in the past, as this subset of veterinarians was considered more likely to recommend a clinical trial in the future, and was the group for whom it would be most valuable to explore the trial features that influence recommendation. Among the respondents, agreement was high that veterinary clinical trials are important for veterinary medicine, and that veterinarians felt comfortable discussing informed consent. This finding would be anticipated for this group, though the response could be different for veterinarians who had not previously investigated or recommended a clinical trial in the past. While this could not be evaluated in the VIN survey, in the VP survey, responses were generally more negative for the item pertaining to comfort discussing clinical trials and informed consent among those that had not recommended a clinical trial in the past than those that had. In human medicine, physician lack of confidence in explaining clinical trials to patients has been noted as a barrier to recruitment.²⁰

The veterinarians in the group that had recommended a trial did not generally believe that their clients would be fearful about participating in a clinical trial, though 78% disagreed or strongly disagreed that their clients were aware of veterinary clinical trials. This is in keeping with findings from a study of pediatricians, where those surveyed believed that

community awareness of randomized clinical trials was low.¹⁴ Among the veterinarians who had recommended a clinical trial to an owner, nearly 50% agreed or strongly agreed that veterinarians should recommend clinical trials to owners as often as possible, while 34% agreed that veterinarians should recommend clinical trials only to select clients. This suggests that even among those veterinarians who strongly believe clinical trials are important and have actively recommended them, some degree of non-invitation of participants occurs.

In human medicine, non-invitation of potential participants for particular studies is a considerable issue for recruitment even among groups of clinical trial investigators.²¹ Reasons given for non-invitation in trials have included concerns about characteristics of the project or protocol, perceptions of the patient or family, and anticipated refusal to participate. Protocol concerns include the involvement of invasive procedures, including blood draws, the inclusion of a placebo group and randomization.^{2,14,22} In our study, veterinarian respondents generally did not indicate that any of the trial features queried would make them significantly less likely to recommend a clinical trial, with the exception of a lack of funding in the VIN survey, and invasive sampling and rare disease in the VP study. However, shifts toward negative influence were seen for trials involving a new treatment for a condition with a currently successful therapy, and those that had invasive sampling or interfered with daily activities, with 25%, 22.7%, and 26.8% of VIN survey respondents, respectively, indicating that these features would make them less likely to recommend a clinical trial. Respondents did believe that the chance of the pet being in a placebo group was important to clients in

considering a clinical trial (75% selected important or extremely important) but the presence of a placebo group in the veterinarian's decision to recommend the trial was not examined.

Nearly 30% of respondents to the VIN survey stated that they did not generally learn of clinical trials in their area. In addition, among the most common reasons given for not investigating or recommending a clinical trial was lack of awareness of clinical trials and the process for enrolling a patient in a clinical trial. Similarly, distance to clinical trial centers was selected as a reason for not investigating enrollment in a clinical trial by over 30% of VIN respondents, and by nearly 70% of those that had investigated a clinical trial but not recommended it. However, we did not inquire about how respondents defined "clinical trial center," which could include referral hospitals, universities, or private clinics. Future work should incorporate further questions about how and where veterinarians believe clinical trials are conducted, as this may represent an educational opportunity. In clinical trials in humans, proximity to a clinical trial center is associated with increased likelihood for referral.²² In the current study, positive influences on participation included that the trial was run by a respected investigator, was sponsored by an academic institution, and that clinicians would be informed of the trial results. This suggests that increased communication with clinicians could increase recruitment to clinical trials.²³

Factor analysis showed that certain features were associated with a higher or lower likelihood of investigating a clinical trial. Higher scores on two factors (Factors 2 and 3) were associated with increased likelihood of investigating enrolling a patient in a clinical trial. The features that were most positively influential in scores for Factor 2 included several

related to the doctor-client relationship, while those for Factor 3 included features believed to be of importance to the client. Higher scores on Factors 2 and 3 indicate relatively lower scores for those features that were most negatively influential, including distance to the trial center and number of re-checks (Factor 2) and logistical features, particularly those related to time (Factor 3). Features related to time were also positively influential in Factor 4 scores, where a higher score was associated with a decreased likelihood of investigating enrolling a patient in a clinical trial.

The issues related to time required for the study are important to acknowledge in veterinary general practice, where time is often short in busy practices, and the time to investigate trials, discuss trials with owners, and pursue enrollment may be a strain when these avenues are not clearly outlined. This has been noted as an issue in studies of clinical trials in humans as well.^{4,24-26} In a study of oncologists, it was found that those who spent more time dedicated to patient care rather than research discussed clinical trials with their patients less than those with a higher research focus.²⁴ A study of pediatricians found that longer time in initial interview/discussion with potential clinical trial participants led to lower refusal rates, a practice that is not always feasible in busy general practices.¹⁵

The respondents' ratings of importance to clients of particular items showed that several features were believed to be very important to clients in considering clinical trial participation for their pets. The pet having a condition that could benefit from the trial received the highest ratings of importance, with 71% of respondents giving it the highest importance rating. This is similar to studies in humans,²¹ and also similar to a study of cat

owners' views on participation in clinical trials, where 73% of respondents gave this item the highest importance rating (out of five).⁷ Veterinarian endorsement of the trial was also rated as important, though only 33% of respondents gave this the highest importance rating. This is likely an underestimation, as physician recommendation has been cited as a critical feature of clinical trial participation in people, and in the study of cat owners, 56% gave the highest importance rating (out of five) to the item "My veterinarian recommended the trial". Future work to compare the views of pet owners (including dog owners) to the views of veterinarians would be useful to evaluate the commonalities (and gaps) between importance ratings.

There are several limitations of the current study. Overall participation in the survey was low, given the number of veterinarians in small animal general practice, however participation was similar to other survey studies.²⁷ This makes generalization of the findings difficult, however, findings from this study are similar to the research conducted among physicians in human medicine, particularly pediatric medicine. Also, internet-based survey studies are subject to volunteer bias, where the views of those that participate might not represent the views of those that did not volunteer to complete the survey. Several items on the VIN survey were asked only of respondents who had recommended a clinical trial, so it is not known how veterinarians who had not recommended a clinical trial would have rated some of the clinical trial features. Given differences in the survey instruments, it was not possible to combine survey items to compare responses from veterinarians in different countries. In the future, an effort to obtain international participation on the same survey instrument delivered in the same manner would help evaluate these potential differences.

This survey was also limited to small animal practitioners, while clinical trials also take place in equine, production animal, and zoological species, among others, and future work should be done to investigate views among practitioners in these areas and specialties. Despite these limitations, this survey provides a first look at veterinarian attitudes about clinical research, and a basis for follow up studies. Several studies in human medicine have used small groups of physicians and more in-depth discussions to explore attitudes toward clinical research.^{14,23,28} While these studies generally have far fewer participants, the ability to ask follow-up questions can provide details not accessible through a survey tool, even with free text options, and is a method that could be explored in veterinary medicine. The current study also provides some indications of factors that are potentially modifiable for increasing veterinarian recommendation of and participation in clinical trials.

The results of this study identify trial features deemed important to veterinarians, and those that veterinarians believe are important to their clients. The results indicate the need for centers to better inform small animal practitioners about clinical trials. This could be accomplished through the development of a web-based searchable clinical trials registry and other communication tools. Multi-site trials, which reduce distance to trial centers might also increase recommendation and successful enrollment of patients into trials. In addition, development of educational materials that can streamline the clinician's discussion of a clinical trial could help to provide information, raise awareness, and relieve some of the time pressure that exists with clinical trial recruitment. Our results also suggest that even when veterinarians are aware of a clinical trial, they may not invite participants if they do not believe in the value of the study or distrust certain protocol or patient care features. Future

work should investigate the impact of interventions, such as increasing clinician involvement in study design and communication of results, on recruitment efforts. In the VP survey, 56% of respondents disagreed or strongly disagreed with the statement, “I believe I received enough education on clinical trials during veterinary school,” while only 12.6% agreed or strongly agreed with that statement. This finding identifies a shortcoming in current veterinary curriculum that could be addressed through increased education of veterinary students. Investigation of the views of first-year veterinary students with those completing their clinical year, could help identify the adequacy of their education about clinical trials during their veterinary training.

^a JMP Pro 11.0 (SAS, Cary NC, USA)

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Chapter 3 : Caregiver Placebo Effect and Mitigation Strategies

Introduction to Chapter 3

The next substantial hurdle in the ability to study efficacy of therapies for DJD-associated pain is the caregiver placebo effect. This chapter begins with a quantification of the caregiver placebo effect in studies of analgesics for DJD-associated pain in cats (Section 3A). Following this, sections 3B and 3C will introduce two methods for mitigation of the caregiver placebo effect. The first is through refinement of outcome measures, and reports the results of a study that built on refinement and validation of the Feline Musculoskeletal Pain Index. The second introduces a novel method for study design in veterinary medicine that allows for the return of clinical signs (deterioration following analgesic therapy) to become a clinically relevant study endpoint.

**Chapter 3a. Quantification of the Caregiver Placebo Effect in Trials of Analgesics for
Cats with Naturally-Occurring Degenerative Joint Disease and Associated Pain**

Introduction

The placebo effect in clinical trials has been extensively studied and reviewed in the human literature.¹⁻³ It has been defined as a beneficial response to an inert treatment, a response that exists for reasons unrelated to the actual treatment given, but more to the milieu (context) in which the treatment is provided and the experience and expectations of the patient.⁴ Context in which the placebo is administered as well as specific traits of the patient have been shown to differentially affect the placebo response.⁵ In studies that include caregiver ratings, the placebo effect may be parsed into two separate, but related, phenomena: the effect of the placebo on the patient receiving the placebo, and the effect of the placebo on the ratings of outcomes provided by caregivers and family - the placebo-by-proxy effect.^{6,7} Interestingly, these placebo-by-proxy changes in ratings can, in fact, directly influence the subject actually receiving the placebo, thereby enhancing the placebo effect.

In studies of analgesics for chronic pain conditions, the placebo effect has been shown to be powerful enough to improve self-ratings of pain and function in a substantial percentage of trial participants. A recent analysis found that, globally, this placebo effect has been increasing over the past 20 years, driven largely by an increase in the placebo effect in trials conducted in the United States.⁸ This effect has made it difficult for new analgesics to show efficacy above a placebo, considered the gold standard for treatment approval in the United States, and hindered the development and marketing for analgesics that could be useful for patients with chronic pain. A myriad of factors influence the placebo response in human clinical trials; these include trial design features, features specific to the treatment and to the route of administration, and patient-specific features such as baseline severity⁹ and

dispositional optimism.^{10,11} Trials that highlight the cost of the treatments or value to the patient may inflate the placebo response.^{12,13} The effects of expectation are significant, for example, adverse event reporting in placebo arms of randomized clinical trials has been shown to vary by type of active medication under study.^{14,15} In designing a trial, investigators must be careful to consider the effects of the placebo response in their protocols, and adjust accordingly.

While the placebo effect can be detrimental in the context of a clinical trial, there are potential benefits to the placebo effect in clinical practice. A placebo, typically physiologically inert, offers the potential to impact self-ratings without the risk of adverse events. Research in humans shows that placebo analgesia involves endogenous antinociceptive systems as well as brain areas involved in pain modulation,¹⁶ meaning that clinically, placebos can be truly beneficial to patients. Several areas in human medicine have begun to take advantage of the placebo effect to enhance treatment response.

However, the placebo effect becomes more problematic when using *proxy* measures of pain or function. While the placebo-by-proxy effect in trials of anti-depressants can bolster the human support network around a patient, and contribute to an increased, potentially beneficial placebo effect to the patient themselves, inflated ratings from caregivers, when valued over self-report, may also mean that patients are not receiving needed, effective treatments. This is particularly true in research using populations unable to provide self-report for outcomes, including some pediatric, elderly, and disabled populations. Clinician ratings as well as caregiver ratings have been shown to reflect a placebo effect in analgesic

trials, and it has been shown that observer, including clinician, ratings show an increased placebo effect size relative to patient self-report.¹

In veterinary medicine, by necessity all subjective outcome assessments and clinical metrology instruments (questionnaires) are completed by a proxy. This makes understanding the placebo effect in proxy assessments even more critical. The placebo effect in this setting not only thwarts our ability to evaluate novel therapeutics in a trial setting, but also means that pets may be exposed to treatments that are ineffective or have unfavorable risk: benefit profiles.

The placebo effect in veterinary trials has been described in the past,¹⁷⁻¹⁹ and has been referred to as the ‘caregiver placebo effect’ in contrast to the placebo-by-proxy effect. In this context, the caregiver placebo effect refers strictly to improved ratings of outcomes in companion animals in the absence of improvement on objective measures. The bases for this effect are likely multi-factorial, and include a desire for the trial to work, a wish to please the investigator, the natural waxing and waning of symptoms in many chronic diseases, and the “better care’ effect, where access to better health care and more follow-up can improve caregiver ratings on subjective measures. While it is yet to be studied in veterinary medicine, one could postulate that caregiver ratings could also be influenced by caregiver features including suggestibility, empathy toward animals, and optimism, among others. To our knowledge, dispositional optimism has not been evaluated for its role in caregiver ratings of placebo responsiveness. In one study in pediatric oncology, it was shown that caregiver

(parental) dispositional optimism was associated with more optimistic prognostic estimates, but this was not evaluated in response to any treatment.²⁰

As an additional consideration to the caregiver placebo effect, it is unknown in veterinary medicine whether there is any benefit of the placebo-by-proxy effect on animals. For many conditions afflicting veterinary species, benefit is unlikely. However, for conditions including treatment for chronic pain, anxiety, or behavioral calming, changes in the caregiver behavior and/or caregiver-pet interactions that result from their belief that their pet is receiving an active medication, could result in actual changes in the behavior of the pet resulting from a real modulation of the condition, e.g. pain relief. Such caregiver behavior may include additional attention paid to the pet, a heightened awareness of the pet's behavior and needs, or an increase in the interactions between the pet and caregiver. If an objective measure of improvement was seen, in the absence of an active treatment, and this tracked with a positive caregiver placebo effect, this may be an indication the animal could be deriving some benefit from this caregiver belief - a real placebo-by-proxy effect. Increased activity, in the form of exercise, has known beneficial effects on pain associated with arthritis in humans²¹ and increased interactions with a pet during a study, as might result if the caregiver thought the animal was receiving active analgesic treatment, could result in increases in exercise undertaken by the pet, and so effects on pain. If it were true that such positive and/or increased interactions with pets is beneficial in terms of pain relief, it would indicate that outside of a clinical trial, there would be an advantage in supporting these ancillary effects, however these potential effects are not understood, and have yet to be evaluated in animals with degenerative joint disease or osteoarthritis (DJD/OA). Figure 1

illustrates the possible relationship between the caregiver placebo effect, placebo-by-proxy effect, and outcome.

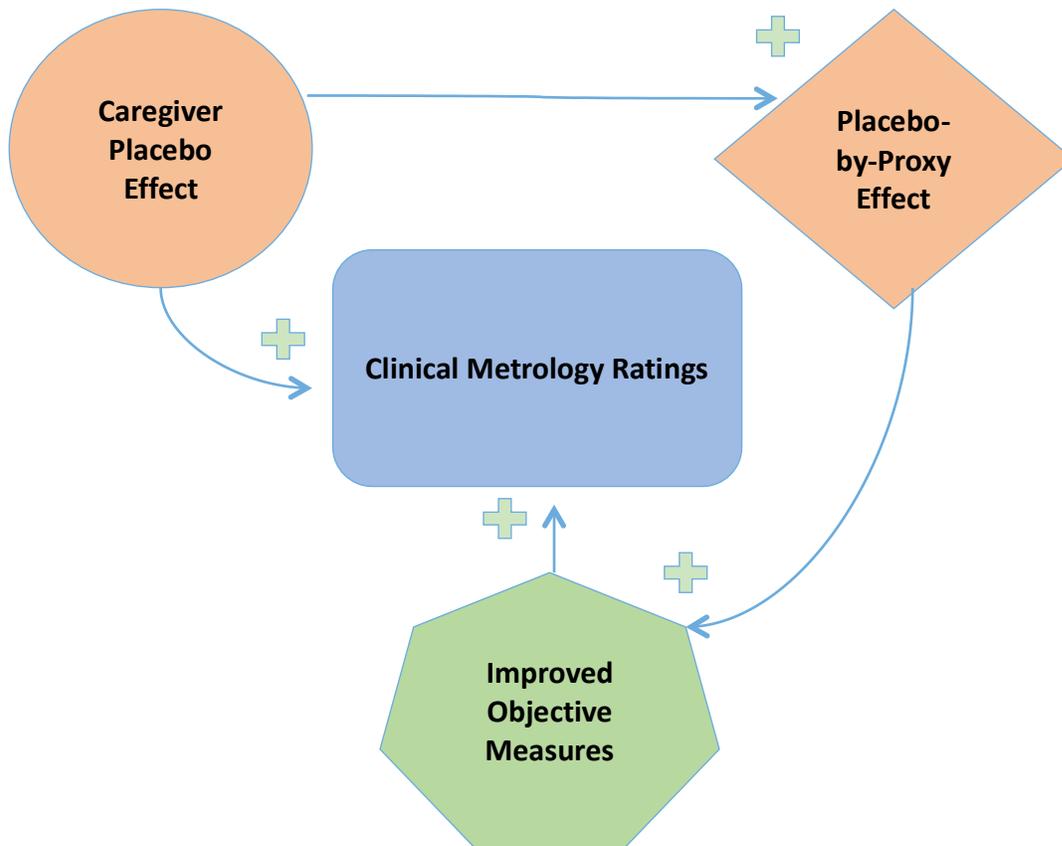


Figure 1. Hypothetical relationship between the caregiver placebo effect, the placebo-by-proxy effect, and outcome.

The objectives of the current study are to quantify the placebo effect in studies assessing therapies for DJD/OA associated pain in client-owned cats. Further, we wish to understand whether there are features of the cats or owners that affect the placebo response. Additionally, we aimed to begin to explore the effect of owner optimism on the caregiver placebo effect, and set the stage for future work to understand the owner characteristics that

affect placebo responsiveness. Finally, we aimed to quantify the percentage of cats that show a potential placebo-by-proxy effect, as defined by success on the subjective measures as well as success in objectively measured activity. This study will identify areas for future research into features that effect caregiver placebo and placebo-by-proxy effects.

Materials and Methods

Five studies using client-owned cats and a combination of subjective and objective outcome measures have been performed in the Comparative Pain Research Laboratory at the North Carolina State University's College of Veterinary Medicine, allowing easy access to the data for analysis for this study. A search for additional studies was performed using Web of Science, CAB Abstracts, and PubMed for (feline or cat) and (arthritis or osteoarthritis or degenerative joint disease) and placebo (searches performed on October 8, 2015 and January 19, 2016). Studies were investigated for the use of client-owned cats and subjective outcome measures (questionnaires). Only 1 additional study met the inclusion criteria (the use of client-owned cats and subjective outcome measures in a placebo controlled trial).²² A request for data sufficient to calculate an effect size in the placebo group was requested from the study that met inclusion criteria, but data were not provided.

A brief description of the included studies follows, with each one being listed using the NCSU-designated name:

1. Diet study (2009)²³ – In this study, 40 cats were screened and enrolled in a prospective, double-blind, controlled, parallel arm study evaluating the efficacy of a formulated supplemented diet in treating cats with DJD/OA associated pain. All

cats were evaluated at Day 0 with an orthopedic exam and radiographs were taken of all joints where pain was detected. Outcome measures included assessments of the cats' ability to perform a set of activities as measured by VAS scale, the Client Specific Outcome Measure (CSOM) Questionnaire (5 items), and accelerometry. In this study, cats were transitioned to the study (n = 20) or control (n = 20) diets from their regular diets over study days 1-7, and remained on their new diets for 9 weeks following complete transition. Activity counts were collected throughout the study, and owner assessments were completed on Days 0, 14, 42, and 70 of the study (after 1, 5, and 9 weeks on the diets). One cat in the placebo group was removed from the analysis as there were no Day 70 owner assessments completed. Aside from general health, eligibility requirements included a CSOM score of ≥ 7 (of 20, with higher scores indicating greater impairment), pain on manipulation of at least one axial segment or appendicular joint that has radiographic evidence of DJD.

2. DQ study (unpublished, 2011)– In this study, 59 cats were screened and enrolled in a prospective double-blind, placebo controlled, parallel arm study evaluating the efficacy of a nutraceutical (glucosamine/chondroitin and avocado/soybean unsaponifiables) in cats with DJD/OA associated pain. All cats were evaluated at Day 0 with an orthopedic exam and radiographs were taken of the painful joints. Outcome measures included a version of the Feline Musculoskeletal Pain Index (FMPI), the CSOM (5 items), and accelerometry. In a unique study design, all enrolled cats were administered placebo (blinded) during the first period, between Days 0 and 14. After this period, cats then received either daily placebo (n = 30)

or daily active treatment (n = 29) for 6 weeks with activity counts collected throughout the study and owner assessments completed on Days 28, 42, and 56 of the study (after 2, 4, and 6 weeks of treatment). Aside from general health, eligibility requirements included a CSOM score of ≥ 7 (of 20, with higher scores indicating greater impairment), pain on manipulation of at least one axial segment or appendicular joint that has radiographic evidence of DJD.

3. FMPI study (2012)²⁴ – In this study, 25 cats were screened and enrolled in a prospective, placebo-controlled crossover trial to determine the efficacy of a non-steroidal anti-inflammatory medication for the treatment of DJD/OA associated pain. All cats were evaluated at Day 0 with an orthopedic exam and radiographs of each appendicular joint and axial spinal segment. Outcome measures included a version of the FMPI, the CSOM (5 items), and accelerometry. All cats underwent a 2-week baseline period during which activity data were collected, and were then randomized to receive daily meloxicam followed by daily placebo, or daily placebo followed by daily meloxicam with a 2-week washout between each 2-week treatment period. Activity counts were collected throughout the study, and owner assessments were completed on Days 0, 14, 28, 42, and 56. For evaluation of the placebo effect, only the first treatment period of the study was included. Aside from general health, eligibility requirements included a CSOM score of ≥ 7 (of 20, with higher scores indicating greater impairment), pain on manipulation of at least one axial segment or appendicular joint that has radiographic evidence of DJD.

4. Low-dose study (2013)²⁵ – In this study, 58 cats were screened and enrolled in a prospective, placebo-controlled crossover trial to determine the efficacy of a low-dose of a non-steroidal anti-inflammatory medication for the treatment of DJD/OA associated pain. All cats were evaluated on Day 0 with an orthopedic exam and radiographs of each appendicular joint and spinal segment. Outcome measures included a version of the FMPI and the CSOM (3 items), and accelerometry. All cats underwent a 2-week baseline period during which activity data were collected, and then were randomized to receive daily meloxicam followed by daily placebo, or daily placebo followed by daily meloxicam with a 3-week washout period between each 3-week treatment period. In a design change from the FMPI study, the 3-week washout period was blinded, with all cats receiving daily placebo, unbeknownst to owners. Effects of this study design are discussed further in a later section of this chapter. Activity data were collected throughout the study, and owner assessments completed on Days 0, 14 (baseline), 35, 56, and 77. For evaluation of the placebo effect, only the first treatment period of the study was included. Aside from general health, eligibility requirements included a CSOM score of ≤ 5 (of 12, with lower scores indicating greater impairment), pain on manipulation of at least two axial segments or appendicular joints that have radiographic evidence of DJD.
5. NV-02 study (unpublished, 2015) – In this study, 34 cats were screened and enrolled in a prospective, placebo-controlled, parallel arm trial to determine the pilot safety and efficacy of a single dose an anti-nerve growth factor monoclonal antibody for the treatment of DJD/OA associated pain. All cats were evaluated on

Day 0 with an orthopedic exam and radiographs of each appendicular joint and axial spinal segment. Outcome measures included a version of the FMPI and the CSOM (3 items), and accelerometry. All cats underwent a 2-week baseline period during which activity data were collected, and were then randomized to receive a single injection of NV-02 (n=23) or placebo (saline, n = 11). Cats were then followed for 9 weeks post-injection, with activity data collected throughout, and owner assessments completed on Days 0, 14, 35, 56, and 77. Aside from general health, eligibility requirements included a CSOM score of ≤ 5 (of 12, with lower scores indicating greater impairment), pain on manipulation of at least two axial segments or appendicular joints that have radiographic evidence of DJD.

All cats in the included studies had similar screening procedures and criteria for study entry, comprised of physical, orthopedic, neurologic, and radiographic exams. Each cat received a total pain score (TPain) based on behavioral reactions to palpation and manipulation during the orthopedic exam as previously described.²⁶ Joints that were found to be painful during orthopedic exam were evaluated with radiographs to support the diagnosis of DJD/OA. In the NV-02, BIVI, and FMPI studies, radiographs were taken of all appendicular joints and axial skeletal segments, in order to generate a total radiographic DJD (TDJD) score based on a previously published method.²⁷

Outcome measures for the studies were similar, but not uniform, making direct comparisons possible following some adjustment. All of the studies used a Client Specific Outcome Measure (CSOM) questionnaire, where owners were asked to rate their cat's ability

to perform several individually tailored activities at multiple time points. This outcome measure is commonly used in both dog and cat studies, and owner ratings have been shown to improve with analgesic therapies.²⁸⁻³⁰ Using 3 activities, each scored 0-4, a “success” on the CSOM has been defined as an improvement in total score of at least 2, representing approximately a 16% change in total scale.³¹ This was the CSOM system used in the Low-dose and NV-02 studies. In the earlier studies (Diet, DQ, and FMPI), a CSOM with 5 activities was employed and thus the success criterion was translated to a change in score of at least 4 - a slightly higher, 20% change in total scale.

Objectively measured activity data, collected via accelerometry^{32,33} were collected in each of these studies as previously described using Actical® accelerometers set to collect activity data at 1-minute epochs, with each cat wearing the same accelerometer throughout the duration of the study. Data were summarized as mean activity counts per minute averaged over the baseline period and the treatment periods for each cat. An improvement in mean activity counts per minute of at least 10% over baseline was used to define “success” based on studies showing an approximate increase in activity of ~10% in both dogs³⁴ and cats (unpublished data) treated with an NSAID. For this, each cat acted as its own comparison.

For each study, each cat was classified as a CSOM success or failure and as an activity success or failure based on these criteria, using the time points described in Table 1. For the NV-02 study, a single injection was given, and the time course of the study was longer than the expected duration of action of the therapy. Therefore, definitions of CSOM

and activity success or failure in this study were set at 3-weeks post-treatment. In each study, cats that were CSOM successes, without having an increase in activity were considered to have “improved” due to the caregiver placebo effect. Cats that were CSOM successes and activity count successes were considered to have improved due to other factors. These factors could include, certainly, the natural waxing and waning of DJD-related pain or regression to the mean,³⁵ though the studies attempted to minimize regression to the mean by averaging activity over several days. A contributing factor could include a placebo-by-proxy effect, where owners are paying increased attention to their cats, engaging them more frequently in interactions and play, and thereby increasing both their total activity and the owner ratings of their abilities.

Table 1. Description of study time points used for assessment of caregiver placebo effect.

Study	Time points used for assessment	Comments
Diet	Day 0 and Day 70 (end of study)	Day 0 was used because by Day 14, cats had already been transitioned to new diet and “treatment” started. Baseline activity was the average activity counts over Days 0-7, while activity counts during Days 63-70 were used for comparison.
DQ	Day 0 and Day 56 (end of study)	Day 0 was used as owners believed that treatment could have started, despite all cats being given placebo for the first 2 weeks. Baseline activity was the average activity counts over Days 0-14, while activity counts during Days 49-56 were used for comparison.
FMPI	Day 14 (baseline) and Day 28 (end of first treatment period)	Day 14 was the assessment just prior to the start of the treatment. Baseline activity was the average activity counts over Days 2-13, while activity counts during Days 16-27 were used for comparison.
Low-dose	Day 14 (baseline) and Day 35 (end of first treatment period)	Day 14 was the assessment just prior to the start of the treatment. Baseline activity was the average activity counts over Days 0-14, while activity counts during Days 38-35 were used for comparison.
NV-02	Day 14 (baseline) and Day 35 (first assessment period following single-injection treatment)	Day 14 was the assessment just prior to the start of the treatment. Baseline activity was the average activity counts over Days 0-14, while activity counts during Days 28-35 were used for comparison.

Effect sizes (as Cohen’s *d*) were calculated for placebo and treatment arms of each study using the average score and standard deviation for each outcome measure for the period following treatment vs. baseline. The effect size is a way to standardize the magnitude of difference between two treatments,³⁶ in this case, the placebo and active treatment groups. Cohen’s *d* represents the standardized mean effect, with 99.6% of the values falling between -3 and 3. Generally speaking, when comparing groups, an effect size of 0.8 is considered

large.³⁷ Effect sizes for treatment over placebo were calculated using the change in score from baseline for treatment and placebo groups, and standard deviation for the change in score. Effect sizes were calculated according to the following equations:

Cohen's *d* for treatment over placebo= (Mean for change in treatment group)-(Mean for change in placebo group)/ pooled standard deviation

Cohen's *d* for the treatment or placebo group= (Mean score following treatment/placebo)-(Mean score for baseline)/ pooled standard deviation

For the NV-02 study, a pilot investigation of the effects of dispositional optimism on proxy ratings of improvement was performed. In order to evaluate dispositional optimism, a previously validated scale (the Life Orientation Test-Revised³⁸) was given to owners to complete once, at their initial screening day. This test consists of 10 items, six of which relate to the optimism/pessimism domain, while four serve as fillers so that the objective of the scale is not easily discernable. Respondents are told that there is no "correct" answer to any item, and they should answer as truthfully as possible. They then indicate their level of agreement with each statement on a five-point scale, where the middle value is neutral. Responses are converted to numerical values ranging from 0-4 according to the published key,³⁸ and higher scores indicate higher levels of optimism, though there is no published cut-off for being an "optimist" or "pessimist". The effect of dispositional optimism scores on CSOM success (Y/N) and on belief that their cat received active medication during the placebo period were evaluated using logistic regression. The relationship between dispositional optimism score and change in CSOM score over the placebo period was analyzed using ANOVA.

Study populations were compared using ANOVA with post-hoc testing when an overall effect was found. Further investigation of the factors that were associated with success/failure were analyzed using logistic regression to evaluate the distribution of CSOM success/failure against cat age, weight, study, baseline activity, TDJD score (when available), and total orthopedic exam pain score. Type of study was also investigated by classifying studies as Drug (NV-02, FMPI, and Low-dose) or Non-Drug (DQ and Diet) and distributions of success/failure investigated using Chi-square tests.

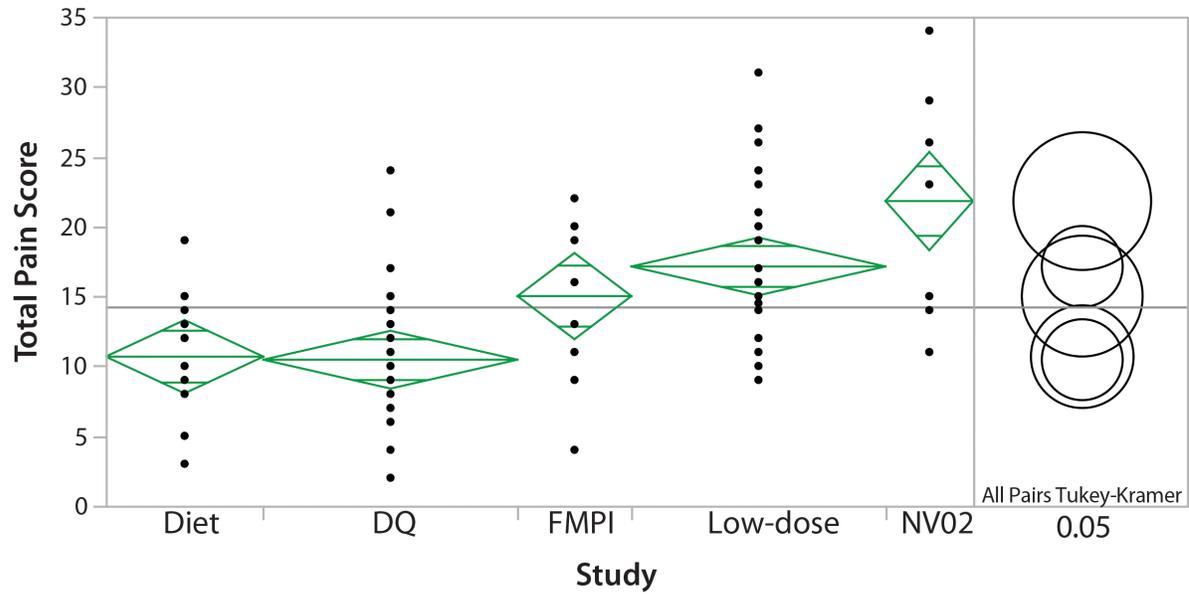
Results

Data from any cat/owner dyads that participated in more than one study were included only for the first study they participated in (n=3). Distribution of the cats in the placebo groups is summarized in Table 2. For evaluation of dispositional optimism, all 11 cats that received placebo in the NV-02 study were considered, regardless of participation in a previous study.

Table 2. Demographic information on the cats by study. No significant differences in distribution between the studies were found for cat age, weight, or sex.

Study	Number of cats	Age (mean +/- SD), years	Weight (mean +/- SD), kg	Sex
Diet	19	10.9 +/- 2.7	6.0 +/- 2.2	45.0% FS/ 55.0% MC
DQ	30	12.2 +/- 4.2	5.5 +/- 1.7	63.3% FS/ 36.7% MC
FMPI	13	11.9 +/- 3.6	5.4 +/- 1.1	76.9% FS/ 23.1% MC
Low-dose	29	12.2 +/- 3.2	6.0 +/- 1.8	51.7% FS/ 48.3% MC
NV-02	10	12.5 +/- 2.0	5.7 +/- 1.4	70.0% FS/ 30.0% MC

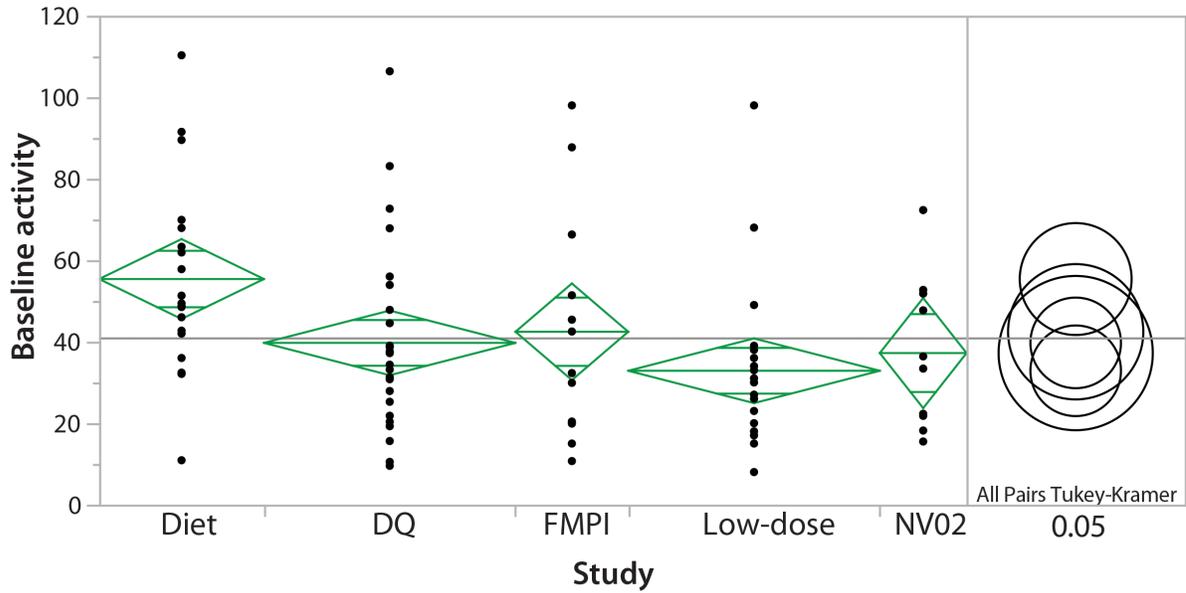
Study populations were not significantly different for cats' age ($p=0.68$), weight ($p=0.89$), or sex ($p=0.27$), but were significantly different for baseline activity ($p=0.015$) and TPain score at baseline ($p<0.001$). Post-hoc testing with Tukey-Kramer showed that TPain score over the study populations were different according to both the investigator performing the study and eligibility requirements of the study (Figure 2). Orthopedic exams were performed in the Diet and DQ studies by one investigator (BDXL), while those in the FMPI study were performed by a second, and those in the Low-dose and NV-02 studies by a third (MEG). As detailed in Table 1, eligibility requirements were more rigorous for the Low-dose and NV-02 studies than for the Diet, DQ, and FMPI. Baseline activity was significantly different between the Diet and Low-dose studies (Figure 3A), with lower baseline activity in the low-dose study (and correspondingly higher pain scores). Overall, the correlation between baseline activity and TPain score was -0.27 (Spearman ρ , $p=0.007$) (Figure 3B).



Study				Mean
NV-02	A			21.80
Low-dose	A	B		17.12
FMPI		B	C	15.00
Diet			C	10.67
DQ			C	10.45

Figure 2. Means and 95% CIs for TPain score by study, with results of Tukey post-hoc testing. Different letters represent means that are significantly different from one another.

A.



Study			Mean
NV-02	A		55.38
Low-dose	A	B	42.44
FMPI	A	B	39.70
Diet	A	B	37.21
DQ		B	32.86

B.

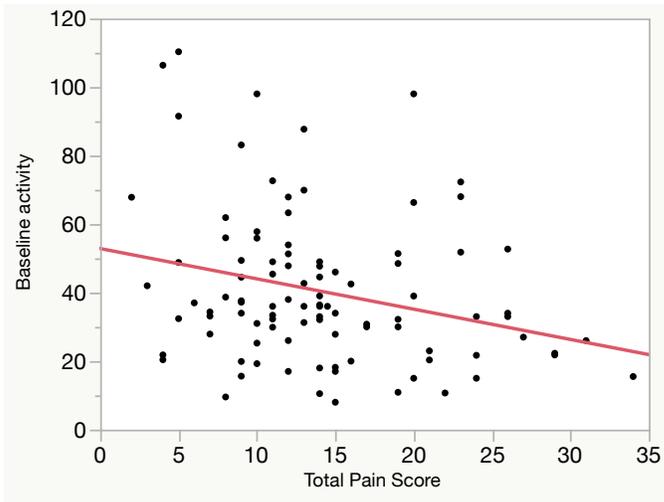


Figure 3. (A) Means and 95% CIs for baseline activity score by study, with results of Tukey post-hoc testing. Different letters represent means that are significantly different from one another. (B) Baseline activity by T-Pain score (correlation -0.27).

CSOM Successes During Placebo Treatment

Improvements in CSOM scores during placebo treatment were seen in all the studies investigated (Table 3). Overall, improvement in CSOM scores that met the criteria for success (CSOM+) occurred in 53% to 73% of the cases. In each study, a percentage of cats improved in activity during the placebo period (Table 3), with a range of 10% to 54% of cats meeting the definition of activity success during the placebo period. The distribution of cats meeting criteria for CSOM success (Overall CSOM+) was not different by study (χ^2 , $p=0.73$), but distribution was different by study for activity success (χ^2 , $p=0.02$) with higher proportions of activity success in the DQ and FMPI studies. Overall, there was a nonsignificant trend for a difference in probability of being defined as an activity success across CSOM conditions (Fisher's exact, $p=0.11$, with $p=0.073$ on a one-tailed analysis; Odds Ratio 2.35 [0.85-6.5]), with 34% of CSOM+ cats being Activity+, but only 18% of CSOM- cats being Activity+.

Table 3. Summary of overall CSOM success during placebo treatment (CSOM+) as well as breakdown of CSOM and activity (Activity+) successes and failures (CSOM- and Activity-) by study. One cat in the placebo group (Diet study) had a malfunction of the accelerometer during baseline, so the breakdown of success/failure for activity excludes this cat. *DQ baseline activity was collected during a period when owners could have believed their cat was receiving active medication.

Study	Overall CSOM+	CSOM+/ Activity+	CSOM+/ Activity-	CSOM-/ Activity+	CSOM-/ Activity-
Diet	14/19 (70.7%)	2/19 (10.5%)	12/19 (63.2%)	0/19 (0.0%)	5/19 (26.3%)
DQ*	22/30 (73.3%)	9/29 (31.0%)	12/29 (41.4%)	3/29 (10.3%)	5/29 (17.2%)
FMPI	7/13 (53.8%)	5/13 (38.5%)	2/13 (15.4%)	2/13 (15.4%)	4/13 (30.8%)
Low-dose	19/29 (65.5%)	6/29 (20.7%)	13/29 (44.8%)	1/29 (3.4%)	9/29 (31.0%)
NV-02	6/10 (60.0%)	1/10 (10.0%)	5/10 (50.0%)	0/10 (0.0%)	4/10 (40.0%)
Overall	68/101 (67.3%)	23/100 (23.0%)	44/100 (44.0%)	6/100 (6.0%)	27/100 (27.0%)

Effect Sizes

Effect sizes for placebo ranged from 1.05 – 1.97. In the treatment arms, effect sizes ranged from 1.09 – 2.06, while the effect size for treatment over placebo ranged from -0.35 (placebo more beneficial than treatment) to 0.74. Effect sizes and 95% confidence intervals (CI) for each study are summarized in Table 4.

Table 4. Effect sizes for the placebo period, treatment period, and treatment over placebo by study. CSOM days used for comparison are also listed.

Study	Effect size (95% CI) (placebo)	Effect size (95% CI) (treatment)	Effect size (95% CI) (treatment over placebo)	Days used for CSOM comparison
Diet	1.93 (1.16-2.70)	1.33 (0.65-2.02)	-0.35 (-0.98-0.28)	D0 vs. D70
DQ	1.71 (1.12-2.30)	1.40 (0.81-1.96)	-0.35 (-0.87-0.16)	D0 vs. D56
FMPI	0.97 (0.16-1.78)	1.40 (0.42-2.37)	0.35 (-0.48-1.18)	D14 vs. D28
Low-dose	1.05 (0.50-1.60)	1.09 (0.53-1.64)	0.09 (-0.43-0.60)	D14 vs. D35
NV-02	1.20 (0.25-2.16)	2.06 (1.34-2.77)	0.74 (-0.02-1.50)	D14 vs. D35

No significant results for the effects of study, cat age, weight, baseline activity, TDJD score, or TPain score on CSOM success in the placebo groups were found. No significant result for study type (drug versus non-drug) by CSOM success in the placebo groups was found (χ^2 test, $p=0.225$).

Dispositional optimism

Dispositional optimism was evaluated in the NV-02 study only. Eleven cats received placebo. Of these, 6 (54.5%) were considered “successes” by improvement in CSOM score at Day 36 while on placebo; owners of 6 cats (54.5%) also believed that their cat received the active treatment, but concordance of these results was only 64%. Dispositional optimism scores for owners of cats in this group ranged from 9-24 (possible range 0-24), however there was no effect of dispositional optimism score on CSOM success ($p=0.695$), nor was there a relationship between dispositional optimism score and change in CSOM score ($p=0.549$).

Dispositional optimism score also did not effect whether owners believed their cats received active medication during the placebo period ($p=0.637$).

Discussion

Overall results of this study show that there is a profound placebo effect in caregiver ratings of improvement in mobility and activity in cats receiving various therapies for DJD/OA associated pain, with approximately 50-70% of cats meeting criteria for success for improved mobility and activity, while receiving a placebo. While the caregiver placebo effect has seemed to decrease somewhat over time, study designs were restructured in an effort to combat the placebo effect, making direct comparisons over time inappropriate. For example, in the FMPI, Low-dose, and NV-02 studies, a 2-week baseline period was included to facilitate both the collection of baseline activity data and to allow a learning period for owners following first exposure to the subjective outcome measures. These changes were made for several reasons. First, many cat owners were unfamiliar with evaluating their cats in the way required by the trials prior to enrolling in a clinical study, and CSOM scores after the baseline period were considered more reliable. In addition, it is known that paying attention to a behavior can change that behavior, or the way that behavior is perceived – a phenomenon known as the Hawthorne effect,³⁹ suggesting that a training period is appropriate. Finally, CSOM scores at Day 0, when owners are actively trying to have their cats deemed eligible for a study, could be artificially worsened as owners might be inclined to evaluate their cat as more in need of treatment in order to meet entry criteria. This is particularly true for the most recent studies (Low-dose and NV-02) where entry criteria were more stringent, and cats needed to be more impaired than required for the Diet, DQ and

FMPI studies. Indeed, if we use the Day 0 (initial screening) day for evaluation of success/failure in the FMPI, Low-dose, and NV-02 studies, our overall placebo effect jumps to 66.7% (FMPI), 86.2% (Low-dose), and 91% (NV-02). This highlights the need to include this baseline period when designing a clinical trial using subjective outcome measures with cats. Preliminary comparison between the types of studies (diet/supplement versus drug/biologic treatment) was not significant, however given the small number of studies available and the diverse nature of the treatments, further work should investigate the effect of treatment type on caregiver placebo effect.

When using a numerical rating scale, as was employed with the CSOM, the difference between individual points at baseline can have a large effect on overall score. The use of visual analog scales, on which owners mark severity along a standardized 100 mm line with two extreme anchors could, in theory, increase the sensitivity and decrease the change from baseline by allowing owners to make more precise assessments of their cats' abilities. In our experience using both types of scales, this has not been shown to be the case, and in the Tuttle et al. analysis of placebo effects in analgesic trials in people, the VAS scale slightly decreased the percentage change from baseline in the placebo groups, but did not increase the ability to detect a treatment effect over placebo.⁸

The DQ study presents a very intriguing study design, with the objective of early exposure of all cats and owners to the placebo in order to mitigate the placebo effect on subsequent caregiver ratings. In theory, this design allows the placebo effect to stabilize after the first two weeks of treatment, followed by further improvement only in the treatment

group. The ideal response would result in a graph such as Figure 4A. However, in this study, what occurred was that overall 47.4% of cats met the criteria for “success” on the CSOM during the initial placebo period. Using change from Day 0, the percentage of “successes” in both the treatment and the placebo group grows over time to 65.5% in the treatment group and 73.3% in the placebo group (Figure 4B). However, if we assume that the Day 14 results represent the proportion of the findings due to the placebo effect, then using that day as our baseline for change, we see that the results now look like Figure 4C.

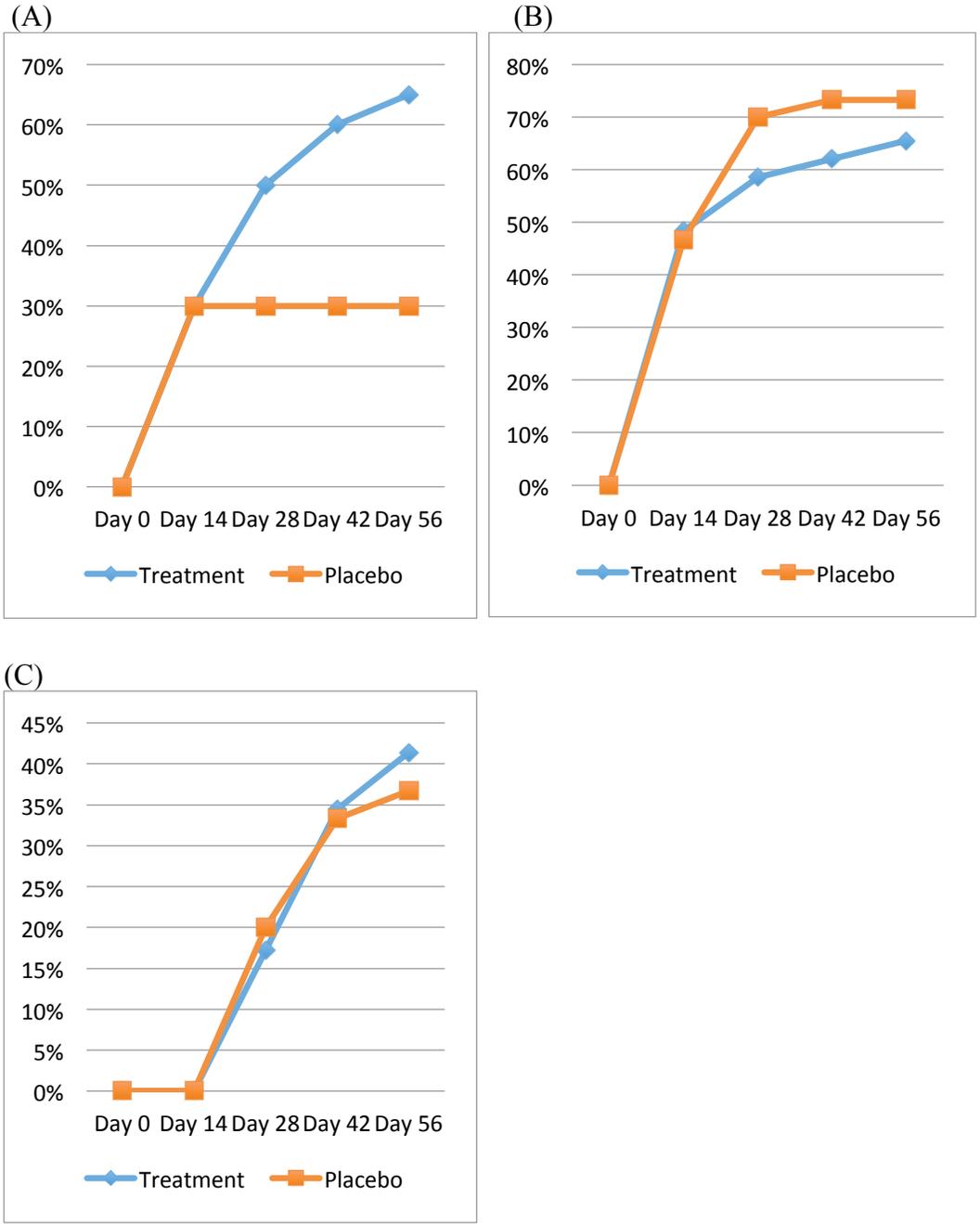


Figure 4. Results from DQ study design. All three graphs show the percent of cases that would be considered CSOM successes by the study day. (A) shows the hypothetical *ideal* results, where the placebo administered during the first two weeks results in a caregiver placebo effect that plateaus after the first two weeks. In this scenario, the treatment group cats would continue to improve in caregiver ratings. (B) shows the *actual* results from the DQ study, where the percentage of cats considered CSOM successes continued to increase in both study groups, negating the ability to discern a treatment effect over placebo. Success defined as a change in CSOM score against the Day 0 score. (C) shows a similar pattern to (B) but success is defined as a change in CSOM score against the Day 14 score.

Unfortunately, as this is a single study of a nutraceutical that failed to produce marked increases in activity in cats, it is not known whether this study design failed to show a difference because the scale could not withstand the placebo effect (i.e. could not increase enough to meet the definition of “success” after already increasing during the placebo period), or because the treatment did not actually produce any beneficial effect. Three of the 59 cats had increased their CSOM score enough during the placebo period that it would have been impossible for them to meet the criteria for success in any successive period, while an additional 3 cats could only meet the criteria for success in a successive period if they had been rated by their owners as completely unimpaired. However, given the finding that by the end of the study, 73% of the cats in the placebo group met the criteria for treatment “success” on the subjective scale, it would be difficult for even the most efficacious therapy to show an effect above placebo using the first day as baseline. The use of the Day 14 as baseline results in a more reasonable improvement, however the placebo effect in this study was remarkably high. A recent study by Sul et al. comparing glucosamine-chondroitin to meloxicam did not find a significant improvement in owner assessments of mobility in the glucosamine-chondroitin group.⁴⁰ The reason for the contrast between these results is not known, but comparator trials may not be subject to the same placebo effect. In addition, the study by Sul et al. was not blinded in the traditional way; savvy owners would readily discern the glucosamine-chondroitin capsules from the meloxicam liquid.

Dispositional optimism

The Life Orientation Test, or LOT-R has been used in multiple studies in humans for evaluating the role of optimism in a variety of outcomes including placebo responsiveness in

trials for dermatologic disease⁴¹ and analgesia.¹⁰ It was recently re-evaluated using item analysis and found to be a valid tool for measuring optimism in people.⁴² Several results point to dispositional optimism, or its converse, pessimism, as potential modulators of placebo responsiveness in analgesic trials, with dispositional optimism thought to be a putative resilience factor against the negative affective consequences of pain. However, this relationship is far from clear, as the factors that influence placebo responsiveness remain poorly understood, and are likely multifactorial, with a single personality trait unlikely to be able to explain all of the variability seen. The influence of situational and environmental context on placebo responsiveness has begun to be explored.⁴³

Despite its limitations, dispositional optimism has been shown to be moderately stable and the reproducibility of placebo analgesia was demonstrated in a study by Morton et al.⁴⁴ While it has not been investigated in the context of caregiver ratings, our hypothesis was that dispositional optimism would be associated with placebo success as defined by improvement in owner clinical metrology instruments while cats were receiving a placebo, but this did not appear to be supported in this small study.

In the NV-02 study, a pilot, there were only 11 animals that received placebo. While this is a very small number of cats and owners, these results do not suggest dispositional optimism is an important source of caregiver placebo response. However, as the dispositional optimism scale is quite brief and easy to administer, incorporation of the questionnaire into future studies would allow further evaluation of the effects of dispositional optimism on caregiver ratings. Further work should also investigate other owner

characteristics that influence ratings on clinical metrology instruments, including empathy⁴⁵ and suggestibility scales. Higher empathy could increase proxy ratings of pain, but could also increase an owner's ability to detect both response to an analgesic as well as a return of clinical signs following withdrawal of a medication.

There are, of course, limitations to the current investigation. To study the placebo effect in trials of analgesics in cats with DJD/OA associated pain requires us to accept certain limitations. We used previously published and accepted criteria for success/failure on clinical metrology instruments completed by owners, while understanding that these criteria could miss the target of a meaningful response. We also adopt a definition of success for improvement in activity counts (as measured by accelerometry) that represents the response seen to medications generally considered effective in cats, as well as other species. The caveat is that increased activity counts do not guarantee decreased pain, yet are used as our surrogate measure. The observation that cats and dogs with DJD/OA appear to have increased activity counts when receiving an analgesic supports the application of this measure.³² In addition, cats with DJD/OA have shown increased spontaneous activity counts when receiving an analgesic, independent of human intervention/interaction (measured at times when humans were not present).^{46,47} This further suggests that activity counts can be a useful surrogate for at least some aspect of pain relief. An additional limitation is that the majority of the studies that have been performed in client owned cats with DJD associated pain have been performed at a single site (NCSU). While the caregiver placebo effect has been demonstrated in client owned cats in several other studies, particularly those in dermatology⁴⁸ and behavior,^{49,50} we are unable to evaluate whether the high placebo effect

reported here is consistent with what would be seen in trials conducted at other centers. The only other study found that used client owned cats with arthritis did show a strong caregiver placebo effect,²² but raw data were not available for comparison. For comparison, however, two open-label trials of analgesics in client-owned cats with naturally occurring DJD/OA found that nearly 100% of owners reported that their cats improved at least slightly while receiving a non-steroidal anti-inflammatory,^{51,52} with 75% reporting moderate to marked improvement in one study.⁵¹ While improvement can be expected with an anti-inflammatory, this level of response is unlikely due solely to the treatment.

It is noteworthy that the caregiver placebo effect appears to be stronger in cats than in dogs. In several recent studies of analgesics for the treatment of OA pain in dogs, a caregiver placebo effect has been noted, with one study noting a caregiver placebo effect occurring in 40% of owner assessments,¹⁹ while another reported a 13.5% (+/- 42.7%) decrease in CMI score during a placebo period.¹⁸ Multiple authors have noted the difficulty in evaluating pain and mobility impairment in cats, and several suggested CMIs have been developed for testing. However, responsiveness testing against a placebo has been restricted to the few studies discussed here. Several putative factors contribute to the challenge of assessing DJD/OA associated pain in cats. Both veterinary and owner awareness of DJD/OA and associated pain in cats lags behind dogs, with the first descriptions of DJD/OA in cats appearing in the literature with a single citation in 1984,⁵³ followed by characterization of the naturally occurring disease beginning in the mid-1990s (vs. the 1960s and 1970s for dogs). Owner interactions with cats in their homes is unquestionably different from owner interactions with dogs, and cats are not asked to perform the same daily tasks as dogs (such

as leash walks, getting into and out of a vehicle, etc.). Features of DJD/OA development in cats also impact the manifestation of the disease. Despite the high radiographic prevalence of DJD,²⁷ many cats with radiographic DJD/OA may not show clinically detectable lameness,⁵⁴ and may be more likely to be perceived as just slowing down or showing decreased mobility as part of normal aging. In a survey of owners of cats diagnosed with DJD/OA, Klinck et al, found that the most commonly noted impairments included jumping, stair use, activity, and gait abnormalities including stiffness and speed.⁵⁵ Owners could easily ascribe these changes to normal aging. General awareness of signs and prevalence of DJD/OA among cat owners is not known.

In the absence of a reliable and clinically applicable objective measure for use in field studies, the strong placebo effect in trials of analgesics for cats makes it more difficult to obtain approval for efficacious therapies. The effect sizes shown in this study exemplify the difficulty with showing effectiveness of a treatment over a placebo when using owner completed CMIs. The converse, of course, is that if owners believe that their cats are improving, when they are not, then cats are not getting the analgesic therapy that they need. While we accept activity counts as a surrogate measure, function may not perfectly equate with pain. In people self-reported function and pain are correlated,⁵⁶ and assessment of pain in the context of physical activity level could improve assessment of function.⁵⁷ For example, a low pain rating for a person sitting on the couch is not equivalent to a low pain rating for that same person while running. However, significant reductions in self-reported pain and increases in self-reported functioning and activity can occur with smaller changes in objectively measured activity,⁵⁸ highlighting one of the limitations of activity monitoring.

The intriguing possibility that there could be some beneficial effect on the cat's activity, from the owner's belief that the cat is on active treatment, bears further exploration. This study found that between 10 and 38% of cats were defined as successes in both CSOM scores and activity during the placebo treatment period. It is not known whether this is a true effect, or whether this represents just the natural course of the disease. Further, even if this is a true effect, it is not known if it would persist outside of participation in a clinical trial – a somewhat artificial setting where owners may be interacting with their cats, observing them and engaging with them more frequently due to the extra demands of the study and the interest of the study investigators. Overall, there was a trend for activity to increase in the cats that were defined as CSOM successes. This could be interpreted as the CSOM working – owners were able to correctly identify the cats that had increased activity. Alternatively, this could be interpreted as a positive expectation having an impact on the cats' behavior. The placebo-by-proxy effect is a relatively new concept,⁷ and studies investigating this effect have a difficult time in differentiating the contributing components in adult populations. A study using a placebo (flower essence) in children with severe temper tantrums found that the number of tantrums children had decreased during the placebo treatment, and attributes this change to a different interaction between the child and the parent that believes their child is getting treatment for their behavior.⁵⁹ While interesting, these results must be interpreted with caution as no objective measures were included. In the context of cats with DJD/OA, a greater understanding is needed of activity patterns in cats with DJD to understand the natural fluctuation of activity over a similar period, outside of a trial setting. This, accompanied by owner questionnaires targeting not only CSOM outcomes but also owner

beliefs, would allow us to further understand this phenomenon, and whether it would have clinical application.

While we continue to strive for clinical metrology instruments that more clearly mirror the effects we see on activity, it will remain important that objective measures of improvement are employed in studies of analgesics. Clinically expedient objective measures currently rely on measures of accelerometry, which are becoming even more accessible as costs continue to decrease for these devices and cats tend to tolerate the collar-mounted monitors well.³³ However, in the future, there is the potential for other biomarkers, validated against activity, to replace accelerometry in clinical trials. Ultimately, a validated measure that could be used by owners that can reliably distinguish between the treatment and placebo would be useful for clinical trials in cats, reducing the cost of the trial. However, outside the context of a clinical trial positive expectations by owners are not necessarily unwelcome. While the positive expectation can be detrimental when a medication or treatment is used that is not analgesic, use of treatments shown to be efficacious in clinical trials could be enhanced in clinical application. As clinicians, we could use the positive expectations of owners to encourage increased interactions, play, and exercise – all of which might increase a cat's activity. Future work should investigate the effect of clinician expectations and priming on caregiver ratings of improvement in cats, as well as more thoroughly evaluate the pattern of activity across days and weeks in cats with DJD/OA and associated pain. Modifications to the analysis of CMIs and innovative study design to combat the placebo effect are discussed in the next sections of this chapter.

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**Chapter 3b. Criterion Validation Testing of Clinical Metrology Instruments for
Measuring Degenerative Joint Disease Associated Mobility Impairment in Cats**

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RESEARCH ARTICLE

Criterion Validation Testing of Clinical Metrology Instruments for Measuring Degenerative Joint Disease Associated Mobility Impairment in Cats

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Data Availability Statement: All data underlying the findings in our study are available in the manuscript, and we have provided a website for downloading the clinical metrology instrument that was used in the study (<http://www.cvm.ncsu.edu/docs/cpr/fmpi.html>) as well as providing this as a supplemental file to this manuscript.

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Abstract

Introduction

Degenerative joint disease and associated pain are common in cats, particularly in older cats. There is a need for treatment options, however evaluation of putative therapies is limited by a lack of suitable, validated outcome measures that can be used in the target population of client owned cats. The objectives of this study were to evaluate low-dose daily meloxicam for the treatment of pain associated with degenerative joint disease in cats, and further validate two clinical metrology instruments, the Feline Musculoskeletal Pain Index (FMPI) and the Client Specific Outcome Measures (CSOM).

Methods

Sixty-six client owned cats with degenerative joint disease and owner-reported impairments in mobility were screened and enrolled into a double-masked, placebo-controlled, randomized clinical trial. Following a run-in baseline period, cats were given either placebo or meloxicam for 21 days, then in a masked washout, cats were all given placebo for 21 days. Subsequently, cats were given the opposite treatment, placebo or meloxicam, for 21 days. Cats wore activity monitors throughout the study, owners completed clinical metrology instruments following each period.

Results

Activity counts were increased in cats during treatment with daily meloxicam ($p < 0.0001$) compared to baseline. The FMPI results and activity count data offer concurrent validation

was not directly involved in data collection, analysis, decision to publish or manuscript content. The sponsor was given the opportunity to review the manuscript and comment. MEG receives funding support from the National Institutes of Health Ruth L. Kirschstein National Research Service Award (T32OD011130).

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for the FMPI, though the relationship between baseline activity counts and FMPI scores at baseline was poor ($R^2=0.034$). The CSOM did not show responsiveness for improvement in this study, and the relationship between baseline activity counts and CSOM scores at baseline was similarly poor ($R^2=0.042$).

Conclusions

Refinements to the FMPI, including abbreviation of the instrument and scoring as percent of possible score are recommended. This study offered further validation of the FMPI as a clinical metrology instrument for use in detecting therapeutic efficacy in cats with degenerative joint disease.

Introduction

Many adult and geriatric cats suffer from Degenerative Joint Disease (DJD) and associated chronic pain.[1, 2] In the United States, there is no approved medication for the long-term treatment of chronic pain in cats, despite the clear need for such a treatment. Several factors impair the ability to find useful treatment options for cats with DJD, including the lack of clinically-applicable, validated screening tools to identify cats with DJD-associated mobility impairment and outcome measures to evaluate efficacy of therapeutics.

Diagnosis of DJD has historically relied on some combination of veterinary examination, radiographic evidence, and owner input.[3–6] Previously, our group developed an instrument, the Feline Musculoskeletal Pain Index (FMPI),[7–9] to address this need. In the current study, we further investigated the function and use of the FMPI, including its ability to detect improvement using the non-steroidal anti-inflammatory, meloxicam, as a therapeutic intervention.

Meloxicam is used commonly in veterinary medicine for the treatment of DJD-associated chronic pain in dogs.[10–12] Meloxicam is currently approved in Europe for use in treating chronic pain in cats, but has not been approved for this use in the United States. There have been several suggestions from open label studies that lower doses of meloxicam than that approved in Europe are effective in the management of feline DJD-associated pain.[13, 14]

The purposes of the current study were two-fold: to evaluate the effectiveness of a 'low dose' of oral meloxicam to improve mobility in cats with DJD-associated chronic pain as measured by actimetry (a non-invasive method to monitor activity), and to further refine and validate the FMPI and other outcome measures. The additional outcome measures included a clinical metrology instrument we have termed the Client Specific Outcome Measure (CSOM),[15] fashioned after the Cincinnati Orthopedic Disability Index described for use in dogs,[16] and owner assessments of quality of life and temperament. Recently, we reported that owners may be better able to discriminate withdrawal of active medication than withdrawal of placebo, and that return of clinical signs following a treatment period might be a useful measure of efficacy.[17] This approach was also included in the data presented here.

We hypothesized that a low-dose of oral meloxicam given once daily would increase activity counts in cats as measured by actimetry and that activity counts would significantly decrease following withdrawal of meloxicam. Further, we hypothesized that the FMPI and the CSOM would respond to treatment, and/or withdrawal of treatment, and show criterion validity with the actimetry measures. Specifically, we tested the responsiveness (ability to detect the effect of an analgesic treatment and ability to detect the withdrawal of an analgesic) of the FMPI and CSOM in a double-masked, cross-over, placebo-controlled study and concurrently evaluated

their criterion validity (whether the changes detected by the instrument correlated with an objective measure of altered mobility) by objectively measuring changes in activity counts in cats with DJD-associated pain.

Materials and Methods

This study was approved by the Animal Care and Use Committee (Protocol # 11-102-O), at North Carolina State University College of Veterinary Medicine (NCSU-CVM), and written owner consent was granted in each case following verbal discussion of the study. The reporting of data follows the CONSORT guidelines.^[18]

Study Design

This was a randomized, stratified, double masked, placebo controlled, crossover clinical trial. Outcome measures included changes in owner ratings as well as changes in activity (as measured by actimetry) after having been treated with the active drug or placebo. Additionally, adverse events and clinical pathology were outcome measures.

Animals

Potential study subjects were identified from clinic records by area primary care veterinarians, or were self-referred by owners that had seen advertisements for the clinical trial. Animals enrolled in the study were all client owned animals with naturally occurring chronic musculoskeletal disease.

Inclusion and Exclusion Criteria

Cats were eligible to participate in the study if they had a qualifying degree of owner-noted mobility/activity impairment (described in the next section), evidence of pain during manipulation of at least two joints or spinal segments during veterinary orthopedic evaluation (described below under *Orthopedic evaluation*), and radiographic evidence of degenerative joint disease in at least two of the painful joints or spinal segments (described below under *Radiographic evaluation*). Cats also had to be greater than 1 year of age and weigh more than 1 kg (2.2 pounds). Predetermined exclusion criteria for all cats included the presence of suspected or diagnosed infectious diseases, symptomatic cardiac disease, immune-mediated disease, neoplasia, inflammatory bowel disease, urinary tract infection, hyperthyroidism, and diabetes mellitus. These conditions were ruled out by careful review of the medical records, owner history, physical examination, complete blood count (CBC), serum biochemistry panels, and urinalysis. Cats with chronic kidney disease (CKD) up to and including IRIS stage 2 ^[19] were eligible to enroll, provided the disease was stable as determined by repeated serum biochemistry panels and urinalyses. Cats with CKD of IRIS stage 3 or 4 were excluded from the study.

Owner Evaluation of Mobility Impairment

During the screening interview, owners were asked to identify three activities that their cat showed impairment in performing. All interviews were carried out by either the study veterinarian (MG) or the study research technician (AT) who worked together to ensure uniform client interviews. Suggestions of types of activities were given to owners (e.g., running, jumping up to a specified height, moving up or down stairs) but owners were encouraged to generate activities specific to their cat. The investigator recommended that the activity be something that the owner felt the cat would be able to perform again if pain was controlled. These three items were then used to construct the Client Specific Outcome Measures (CSOM) assessment

described below, and used for future assessments if the cats were successfully enrolled. Owners rated their cat's ability to perform each activity on a Likert type scale ranging from 4 (No Problem) to 0 (Impossible) with intermediate values of 3 (Mild difficulty), 2 (Moderate difficulty), and 1 (Severe difficulty). Owner ratings were converted to numerical scores, and total CSOM score represented the sum of these three scores with a possible range of 0–12. In order to ensure that cats enrolled in the current study had moderate to severe activity impairments, as rated by their owners, cats were eligible for inclusion if they received an owner-rated score of ≤ 5 on Day 0. Owners completed the CSOM without knowledge of the cut-off for inclusion.

Orthopedic Evaluation

Orthopedic evaluations were carried out as previously described,^[9] and were performed by a single veterinarian (MG) following pre-study training.^[1, 7] Briefly, every joint and axial skeletal segment (cervical, thoracic, lumbar, and lumbo-sacral) was palpated and manipulated to evaluate for signs of pain and instability. As described in ^[9], the pain response for each joint was graded on the following scale: 0 = no resentment; 1 = mild withdrawal, mild resistance to manipulation; 2 = moderate withdrawal, body tenses, may orient to site, may vocalize or increase vocalization; 3 = orients to site, forcible withdrawal from manipulation, may vocalize, hiss, or bite; and 4 = tries to escape or prevent manipulation, bites or hisses, marked guarding of site. A total pain score was calculated as the sum of all the individual appendicular joint and axial skeletal segment pain scores.

Radiographic Evaluation

Cats meeting eligibility criteria for owner-noted mobility/activity impairment and pain on orthopedic evaluation were sedated using a standard protocol and orthogonal radiographs were taken of every joint and spinal segment. Radiographs were reviewed for the presence of degenerative joint disease as previously described ^[7] by a board-certified veterinary radiologist masked to the presence or location of pain.

Randomization Method

The cats were randomly allocated to one of two treatment sequences (meloxicam followed by placebo [PDPP Group], OR, placebo followed by meloxicam [PPPD Group]), which were assigned to clinical cases according to predetermined randomization tables. The first period for all cats was placebo (P), followed by either meloxicam or placebo (D or P), a masked washout period (P), and a crossover period with either meloxicam or placebo (D or P). Randomization was stratified by owner-rated degree of impairment based on total CSOM score. Subjects were categorized as Low Impairment (Level 1) with total CSOM scores of 3, 4, or 5, and as High Impairment (Level 2) with total CSOM scores of 0, 1, or 2.

Masking

NCSU-CVM Pharmacy personnel packaged meloxicam or placebo for administration to each cat. Placebo was identical to drug, minus the active ingredient, meloxicam. Volume of drug or placebo administered was based on the weight of the cat at D0 (enrollment). The volume of placebo was matched to volume of drug for each cat. Packaging for drug or placebo product was identical to prevent identification of treatment group by owners or investigators, and bottles were identified only by the days they were to be administered. Individual bottles for each period were delivered to the owner with both investigator and owner remaining masked to treatment group.

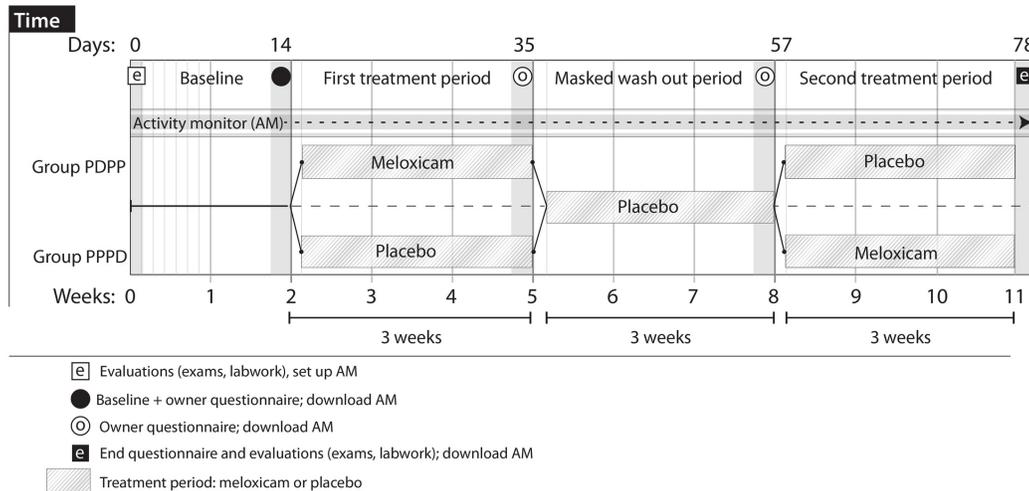


Fig 1. Study timeline and procedures completed at each timepoint.

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Study Timeline

Cats were screened on Day 0 with owner-interview, physical, orthopedic, and neurological examinations, radiographs, and labwork (CBC, serum biochemistry panel, urinalysis, and T4). Eligible cats were enrolled and fitted with an activity monitor (AM; Actical Z, Philips Respironics)³ worn for the duration of the study. Owners were given placebo (unmasked) at 0.07 mL/kg/day to be given orally during the baseline period (Days 1–14). Following Day 14, all future treatments were masked and volume-matched. Outcome measure questionnaires (described below) were completed at regular intervals (Fig 1). During treatment periods, cats received either placebo (0.07 mL/kg/day) or meloxicam (0.035 mg/kg/day). All cats received placebo (0.07 mL/kg) during the masked washout period. The study design was amended to add a second masked washout period (Days 78–99). As only 56% of enrolled cats completed this washout period, data from this period are not discussed here.

Description of Outcome Measures

Safety and adverse events. Blood samples were taken from all the cats at Day 0 (baseline) and Day 78 to evaluate any changes in CBC or serum biochemistry values over the course of the study. Adverse events (AE) were defined as any observations in the cat that were unfavorable and unintended and that occurred during the study, whether or not it was considered to be treatment related. A serious adverse event (SAE) for this study was defined as any adverse event that was either fatal or life-threatening, required professional intervention (by a veterinarian) and considered by the investigators to be clinically serious or caused prolonged or permanent disability or disfigurement. Cats were observed throughout the study for AEs including but not limited to: changes in appetite or thirst, vomiting, diarrhea, changes in urination or defecation frequency and consistency, negative behavioral changes, neurological signs, lethargy or depression. Owners were asked to report any AEs at the time they occurred.

Owners were given appropriate contact numbers for day or evening access to investigators in the case of any AEs or SAEs.

Efficacy outcomes. The primary outcome measures in the study were the AM output and the FMPI and CSOM scores. The secondary outcome measures were Change in QoL (QoL-change), Temperament and Happiness. These owner assessments were conducted at every time point (Days 0, 15, 36, 57, and 78).

Activity monitors. The AM and their use in cats have been previously described. [20–22] Activity monitors were mounted on a neck collar for all cats. Collars either belonged to the cats prior to the study, or were provided by the study for cats that did not have collars. Owners were given the option for a harness mounted activity monitor, but no owners selected this option. Activity monitors were set to collect data with an epoch length of 1 minute.

Owner assessments. Feline Musculoskeletal Pain Index (FMPI): This questionnaire has been used in previous studies of cats with OA/DJD [8] and its development has been described. [7, 9] The version of the FMPI used in the present study differed from previous versions: the option to select ‘greater than normal’ had been deleted because previous analysis collapsed the ‘greater than normal’ and ‘normal’ category into one. The final two questions on the FMPI were converted from a Likert scale to a visual analog scale, and questions asking about ‘overall activity’, and overall quality of life were deleted following previous study results. [8] The FMPI queries owners on their cat’s ability to perform each of 17 activities (rated on a Likert scale from ‘Normal’ to ‘Not at all’) with an option to select ‘Don’t know or Not Applicable’. Two additional items (pain domain) ask owners to rate their cat’s level of pain on a standardized 100mm visual analog scale. Owner ratings were converted to scores ranging from 0–4 for each item with 0 = Not at all and 4 = Normal. Scores on the visual analog scale were calculated by measuring, in mm, from the start (zero point) to the owner’s mark, with 100 indicating ‘no pain’ and then dividing by 25 to align the scales for the entire instrument. The range of possible scores was 0–68 for items 1–17, and 0–78 for the full FMPI. On Day 0, owners completed a weighted FMPI (wFMPI), which asked owners to mark a visual analog scale to indicate the importance of each activity to their cat in addition to indicating their cat’s ability on the Likert scale. This importance scale ranged from 0 = No importance to 100 = extremely important. Importance scores for each item were calculated by measuring, in mm, from the start (zero point) to the owner’s mark. **Client Specific Outcome Measures (CSOM) Questionnaire:** As described above, the CSOM asks owners to identify 3 activities that their specific cat is impaired in performing, and to rate the cat’s level of difficulty in performing those activities on a Likert scale from ‘No problem’ to ‘Impossible’. For each time point after Day 0, owners were also asked to rate the change in their cat’s ability to perform the activity compared to before starting the medication for that period, and these ratings were transformed into numerical scores with Worse = -1, No Change = 0, and Improved = +1 for each activity. CSOM items were each presented in the same order for each cat at each visit. **Quality of Life and Temperament Questionnaire:** This assessment queried absolute Quality of Life and change in QoL, absolute Temperament and change in Temperament, and change in Happiness. Scores from the owner ratings were converted to numeric values as follows: QoL scores (QoL Scores) ranged from 1 = poor to 5 = excellent and a change in QoL (QoL Change) from -2 = much worse to +2 = greatly improved with 0 = no change in QoL; absolute temperament was a nominal scale, while change in temperament (Temp Change) ranged from -2 = much worse to +2 = greatly improved with 0 = no change in temperament. Happiness was rated only as change (Happiness Change), and was converted to a score ranging from -2 = much more unhappy to 2 = much more happy, with 0 = no change in happiness.

Statistical Analysis

Handling of data. Safety was analyzed for all cats enrolled in the study using Day 0 and Day 78 labwork results and adverse events. Cats removed from the study due to AEs were included for safety using their initial labwork, and labwork obtained at the time of the AE. Cats removed from the study due to owner non-compliance were removed from the safety analysis if no follow-up labwork could be obtained. For all safety analyses, data collected on Day 0 were considered baseline. Comparisons to baseline refer to these data.

Efficacy was analyzed in both a parallel design (through Day 57) and a crossover design (through Day 78). Cats were removed from analysis of efficacy in the parallel design if they did not complete the study through Day 57, and from the crossover design if they did not complete the study through Day 78. For all efficacy analyses, data collected on Day 15 were considered to be baseline data. Stratification of the cats by CSOM score group (high impairment vs. low impairment) was incorporated into the randomization, but results were not analyzed separately for these strata. A p-value of 0.05 was considered significant unless otherwise noted.

Group demographics. Groups (PDPP group and PPPD group) were compared for distribution of age and weight using t-tests and gender of the cats using Chi-squared analysis. Groups were also compared on a number of environmental variables using Wilcoxon two-sample tests.

Safety. Paired t-tests were used to compare Day 0 laboratory results with end of study laboratory results (Day 78 for the majority of cases, earlier if cats were withdrawn from the study prior to Day 78). McNemar's test was used to compare distributions on Day 0 and end of study lab work for categorical results. Wilcoxon signed rank test was used to compare Day 0 and end of study results for laboratory variables lacking a normal distribution (using a Kolmogorov-Smirnov test for normality). T-tests were also used to compare end of study results between cats in the PDPP group vs. those in the PPPD group (active medication would have ended 6 weeks prior versus 1 day prior to labwork, respectively in these groups).

Efficacy (parallel and crossover comparisons). **Activity:** Data points (counts) for activity were generated at every minute of every day throughout the study. These activity counts were averaged for each 24-hour day to generate a single data point for each cat over each day and then the mean across a treatment period was calculated to create a single value for each cat for each treatment period. For between group comparisons, the group average activity counts for each day were used. These data were used for initial between group comparisons using t-tests. Due to marked inter-cat variability in activity, activity data were then summarized for each cat as a single value representing the average activity count per minute within a study period. Crossover analysis of improvement for both active treatment periods was performed using ANOVA. Paired t-tests of change in mean activity counts within each treatment group were used to compare within group changes during the active treatment period, and during the period following withdrawal of active treatment for the PDPP group. Paired t-tests were also used to compare between groups for improvement in mean activity count per cat per period by Day 36 over Day 15, and Day 78 over 57, as well as for decreases in activity counts by Day 57 over Day 36 between the PDPP and PPPD groups. **FMPI:** Data from the FMPI were summarized by cat as both raw score (total FMPI points for Q1-17, and Q1-19), and percent possible (%poss) score. Percent possible scores were used to adjust for the fact that some owners could not answer all items (for example, owners without stairs indicated a Not Applicable answer for items pertaining to stairs) and were calculated for items 1-17 and 1-19 by the following equations, respectively:

$$FMPI\%poss\ Score\ Q1 - 17 = \left(\sum Q1 - 17\ scores \right) \div (number\ of\ questions\ answered \times 4)$$

$$FMPI\%_{\text{poss}} \text{ Score } Q1 - 19 = \left(\left(\sum Q1 - 19 \text{ scores} \right) + (Q18 \div 25 + Q19 \div 25) \right) \div (\text{number of questions answered} \times 4)$$

Repeatability (test-retest reliability) of the total score (for both 17 and 19 items) was evaluated in several ways. Spearman's rank correlation coefficients between the Day 0 scores with the Day 15 (baseline) scores were calculated. Wilcoxon signed rank tests were used to check for systematic differences between D0 and D15 scores (if D15 scores were consistently lower or consistently higher than D0 scores). Bland-Altman plots were constructed to compare D0 and D15 scores.

For the parallel design analysis, t-tests were used to analyze improvement during treatment (raw scores and %poss scores on Day 36 minus scores on Day 15) as well as decline during the washout period (termed "deterioration" representing a return of clinical signs following withdrawal of previous medication; raw scores and %poss scores on Day 57 minus scores on Day 36). The FMPI was also analyzed in a success/failure paradigm and these results have been discussed elsewhere.^[17]

For the crossover design analysis, improvement on Day 36 and 78 was analyzed using a general linear model. Both raw and %poss scores were evaluated.

In addition, in an effort to refine this instrument, following analysis of a treatment effect, we analyzed the owner-reported importance scores for each item, independence of items, and determined which items showed significant improvement and significant deterioration in the meloxicam treatment group during Days 15–36 and 57–78 respectively. Kruskal-Wallis test was used to compare FMPI items related to sociability with owner-rated temperament category. A Wilcoxon signed rank test was used to determine the items that showed significant improvement at Day 36 or deterioration at Day 57, and those items that discriminated between the PDPP sequence group and the PPPD sequence group at Day 36 and 57. **CSOM:** For the parallel analysis, total CSOM scores were analyzed using a Wilcoxon rank-sum test for Day 36 (improvement) and Day 57 (deterioration). For the crossover analysis, a general linear model was used to analyze improvement and deterioration in scores. Finally, a success-failure analysis was performed in which the number of cats in each group with a change in CSOM score of ≥ 2 for improvement at Day 36 over Day 15, and ≤ -2 for deterioration at Day 57 over Day 36 was compared using a Fisher's exact test and previously reported. **[17] Quality of Life, Temperament, and Happiness:** For the parallel design analysis, Owner reported change in QoL, Temperament, and Happiness was dichotomized into improved/not (deteriorated/not) and analyzed using a generalized linear model. For analysis within the crossover design, dichotomized results were analyzed with a generalizing estimating equation (GEE).

Results

Enrollment and Distribution of Groups (Meloxicam-Placebo [PDPP] and Placebo-Meloxicam [PPPD] Groups)

A total of 66 cats were enrolled in the study. All cats had lived with their owners for at least 2 ½ months (range 2 ½ months– 16 ½ years; mean 10.25 years). Adequate randomization was achieved with no significant differences found between the groups for age, weight, or gender of the cats (Chi-square test, all ns) (Table 1). No significant differences were found for other demographic variables measured except for the variable "How long have you owned your cat," where the median number of years for the PDPP group was 12, while the median number of years for the PPPD group was 10 ($p = 0.02$; Table 2). This difference, while statistically significant, was not considered clinically significant.

Table 1. Distribution of age, weight, and sex between treatment groups for all cats enrolled in the study.

Variable	PDPP Group		PPPD Group		P-value (t-test)
	N	Mean	N	Mean	
Age in years at start	33	12.6	33	12.5	0.93
Weight (kg)	33	5.58	33	5.63	0.90
	N	% Female	N	% Female	P-value (Chi-square)
Sex	33	48%	33	50%	0.90

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Fig 2 outlines the flow of cats through the study following enrollment. In addition, one cat was removed partway through the study due to development/detection of clinically relevant hypertension with retinopathy (unrelated to study drug). Following stabilization and medical management, this cat was re-enrolled in the study under the same case ID number. Only data from the second enrollment were included in the analysis.

Safety and Adverse Events

Labwork analysis. No significant changes were found between Day 0 and end of study laboratory results. When analyzed by final treatment (meloxicam or placebo), no significant effects seen except in albumin, where cats that were on meloxicam days 57–78 had a significantly higher change (decrease) in albumin from Day 0 to Day 78 ($p = 0.009$). There was not a statistically significant difference in albumin values between groups on either Day 0 ($p = 0.971$) or Day 78 ($p = 0.084$). Overall, two cats had albumin values below the normal range on Day 78, one was mildly decreased at the start of the study, while the other moved from the normal range to below normal (normal range 2.9 g/dL–4.0 g/dL).

Adverse events. All reported adverse events occurring in cats enrolled into the study are summarized in Table 3. Six cats were withdrawn from the efficacy analysis prior to Day 78 due to adverse events. Of these, four cats were withdrawn due to adverse events following meloxicam administration (three cats for vomiting and one for a single seizure), and two cats were withdrawn due to adverse events following placebo administration (one for behavioral disorder/hiding and one for acute kidney injury that was detected during the placebo period following meloxicam administration). Two additional cats had clinically relevant adverse events not reported or noted until the Day 78 exam. One cat had a mild increase in serum creatinine,

Table 2. This table gives the results of Wilcoxon two-sample tests looking for differences between variables that were not controlled for in the treatment group selection.

Variable	PDPP Group		PPPD Group		Statistic	p-value
	N	Median	N	Median		
Number of people living in the house	33	2.0	33	2.0	1126.0	0.78
Number of children under the age of 10	33	0.0	33	0.0	1113.0	0.90
Number of people gone for more than four hours a day	32	1.5	32	1.0	1072.5	0.65
How long have you owned the cat? (years)	33	12.0	33	10.0	905.0	0.01
Number of pets in the house	33	4.0	33	3.0	1066.0	0.61
Number of cats in the house	33	3.0	33	3.0	1161.5	0.47
Number of dogs in the house	33	0.0	33	0.0	1037.5	0.31
Size of house (square feet)	33	2000.0	31	2400.0	1125.0	0.16
Percent of house access for cat	33	100.0	33	100.0	1050.0	0.43
Height easily jumped to (inches)	33	24.0	31	18.0	938.0	0.35

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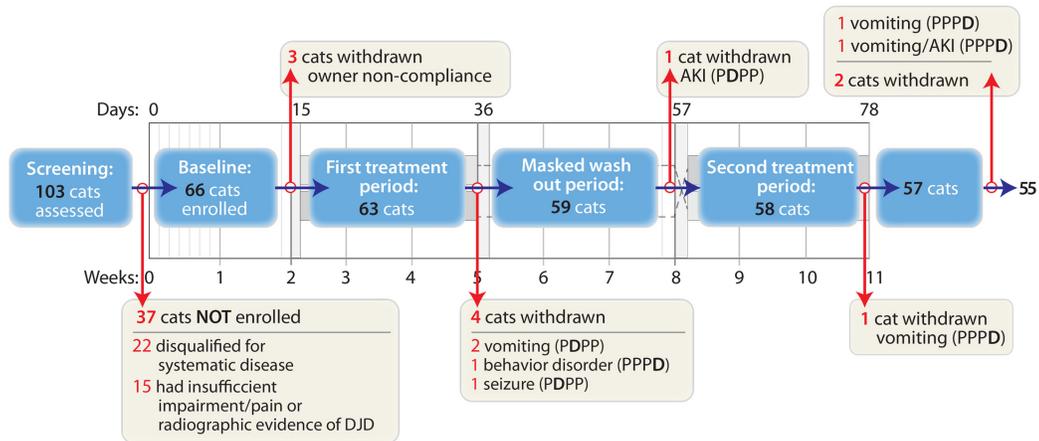


Fig 2. Participant flow diagram. Events occurring at each time point are outlined below. Cats removed from the efficacy analysis were retained for the safety analysis, unless otherwise noted. Cats are designated by the group they were part of (PDPP [meloxicam first] or PPPD [placebo first]).

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while the other had been vomiting for several days prior to examination, and had a marked increase in serum creatinine requiring fluid therapy and hospitalization. These cats were withdrawn from the study with respect to efficacy analysis but are represented in the safety analysis.

Efficacy

Actimetry. When data were averaged across cats, by day and treatment, significant differences between the treatment groups were found for Days 15–36 (first treatment period; $t = 17.7, p < 0.0001$) Days 37–56 ($t = 7.22, p < 0.0001$), and Days 56–78 (second treatment period; $t = -2.35, p = 0.0235$).

Analysis of individual cat patterns of activity counts revealed marked inter-cat variability (Fig 3), suggesting that between group analyses may be less appropriate than within group analyses. The coefficient of variation between cats for the AM data during the baseline period was 50%. For remaining analyses, activity data were summarized as average counts per minute across each treatment period, with one value describing each cat for a given period. Using these data from the baseline period (Days 1–15) and the two treatment periods (Days 16–36 and Days 57–78), there was a strong treatment effect ($p < 0.0001$, ANOVA) and a weak time and sequence effect ($p = 0.088$ and $p = 0.081$ respectively). Cats had increased activity counts when receiving meloxicam, and activity counts tended to be higher during the first treatment period than the second, and higher in the PDPP versus PPPD sequence cohort (Table 4).

Within group comparisons are summarized in Table 5, and show a non-significant increase in activity counts ($p = 0.31$) when meloxicam was administered, but a significant decrease in activity counts ($p = 0.0008$) when active medication is withdrawn in the PDPP cohort. The PPPD cohort showed a significant increase in activity counts when administered meloxicam ($p = 0.0134$), and no change from baseline to placebo, or placebo to placebo.

Comparing between groups for change (improvement) from baseline to the end of period 1, there was no difference between the cats receiving meloxicam in period 1 and those receiving placebo ($p = 0.53$), with both groups increasing in activity counts slightly (Table 6). However,

Table 3. Summary of adverse events reported by owners during the course of the study.

Adverse Event	Placebo (number of cats with sign)/Number of cats removed	Meloxicam (number of cats with sign)/Number of cats removed	Cats removed from study prior to Day78 predominantly due to this AE
Gastrointestinal signs			
Vomiting	11/0	8/3	3
Diarrhea	2	2	0
Regurgitation	1	0	0
Flatulence	0	1	0
Loose stool	1	0	0
Constipation	1	0	0
Behavioral/Neurologic signs			
Head tilt*	1	1	0
Behavioral disorder not otherwise specified	2/1	1/0	1
Seizure	0/0	1/1	1
Urinary/Renal signs			
Nephritis	0	1	NA (end of study)
Acute Kidney Injury	1/1	1/0	1 (1 end of study)
Renal insufficiency	1	1	0
Urinary tract infection	2	0	0
Other Systemic Disorder			
Elevated ALT	0	1	NA (end of study)
Hypertension	0	1	0
Diabetes	1	0	NA (end of study)
Weight loss	1	0	0
Other labwork abnormalities	2	1	0
Other			
Skin lesion	0	2	0
Musculoskeletal disorder (owner concerned about antebrachial swelling)	1	0	0
Nasal discharge/sinus disorder	1	0	0
Aural discharge/infection	0	1	0

Listed are the number of cats with each sign while on placebo and while on meloxicam treatment. In addition, for any adverse event where cats were removed from the study prior to Day78 predominantly due to this AE, the number of cats removed from each group is listed.

* In one case (placebo), the head tilt was transient, in the other case (meloxicam), the head tilt was associated with severe otitis media.

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cats that had received meloxicam during the first period showed a more significant decrease in activity counts during the masked washout (period 3) than the cats that had received placebo in the first period ($p = 0.019$).

Feline Musculoskeletal Pain Index (FMPI). Correlations between D0 and D15 scores for the FMPI with 17 and 19 items were moderately strong ($r_s = 0.72$ and $r_s = 0.73$, respectively). The difference between the means was small (1.55 for the FMPI with 17 items, and 1.57 for the FMPI with 19 items), however Day 15 scores were significantly higher (indicating less impairment) than D0 scores for total FMPI with 17 items ($S = 268.5$, $p = 0.031$) and total FMPI with 19 items ($S = 249.0$, $p = 0.041$). Bland-Altman plots of the D0 and D15 data showed that there were 2 outliers, both cats belonged to same owner. The plots show the majority of cats were scored as being less impaired on D15 compared to D0. There was no obvious association

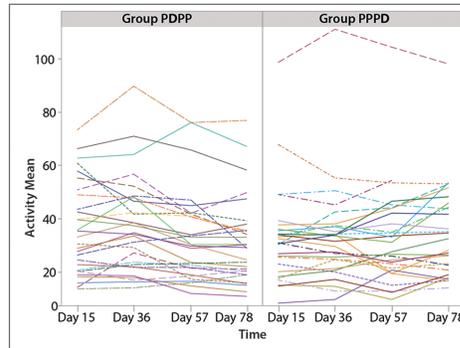


Fig 3. Plots of mean activity counts per day for individual cats in the PDPP (meloxicam first) and PPPD (placebo first) groups. These plots highlight the marked inter-cat variability in activity seen throughout the study.

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between increasing score and an increase or decrease in scatter (Fig 4A and 4B). All points except the 2 outliers lay within +/- 2SDs of the difference.

Results for the parallel analysis are shown in Table 7. A significant difference ($p = 0.048$) in percent possible scores (for items 1–17) was found for deterioration, with the cats that had been on meloxicam during Days 15–36 showing a greater deterioration in %possible scores than the cats that had been on placebo. When raw scores (total of items 1–17 without accounting for percent possible) were compared, no differences between the groups were found for improvement at Day 36 or deterioration at Day 57.

Both percent possible and raw scores were evaluated in a general linear model for improvement on Day 36 and 78 for the crossover analysis, results are shown in Table 8. Deterioration was not evaluated for the crossover as not all cats had a matched post-treatment washout period (Day 99). Percent possible was evaluated for both FMPI Q1-17 and FMPI Q1-19. A statistically significant treatment effect for improvement in percent of possible points was found

Table 4. Summary of analysis of variance of average activity counts per minute per period and least-squares means from the crossover analysis.

Source	Numerator DF	Denominator DF	Type III Sum of Squares	F Value	Pr > F
Treatment	1	52	532.3	20.21	<0.0001
Time	1	52	79.5	3.02	0.088
Sequence	1	94.2	129.6	2.18	0.081
Previous Activity	1	52	48.2	1.83	0.182
Variable	Level	Least Squares Mean			
Treatment	Meloxicam	36.2			
	Placebo	31.8			
Time	Day 36	34.9			
	Day 78	33.2			
Sequence	PDPPP	35.6			
	PPDP	32.4			

Averaging by cat across each period allows for a crossover analysis of improvement for both treatment periods.

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Table 5. Within group comparisons of activity data between periods.

Group: PDPP	Mean Difference	Std. Dev.	T-statistic	P-value
Days 15 to 36 –Days 1 to 15	1.3284	6.8628	1.04	0.3061
Days 37 to 56 –Days 15 to 36	-4.2448	6.1081	-3.74	0.0008
Days 57 to 78 –Days 37 to 56	-1.9101	4.9203	-2.09	0.0458
Group: PPPD				
Days 15 to 36 –Days 1 to 15	0.3497	4.8837	0.39	0.7027
Days 37 to 56 –Days 15 to 36	-0.3653	6.1219	-0.32	0.7503
Days 57 to 78 –Days 37 to 56	2.9776	5.9542	2.65	0.0134

Results from paired t-tests of change in mean activity counts for each cat within each treatment group, Days 1 to 15 versus Days 16 to 36, Days 37 to 56 versus Days 57 to 78. A positive result indicates higher activity counts during the later time period.

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for both 17 and 19 items (meloxicam treated cats had greater improvement than placebo treated cats), however the effect appeared to be stronger for the 17 items. Evaluation of raw scores for FMPI Q1-17 showed an improvement in meloxicam treated cats over placebo treated cats that was significant at the 10-percent level.

There was no significant relationship between average activity count over the baseline period and Day 15 CSOM score ($R^2 = 0.042$; $P = 0.124$) or Day 15 FMPI Score expressed as % possible ($R^2 = 0.034$; $P = 0.166$) (Fig 5A and 5B).

Analysis of owner-rated importance scores showed high variability in responses. Importance scores relative to the overall median importance score across all respondents and all items indicated that items 1, 6, 7, 10, 11, 13, 14, 15, 16, and 17 were rated as relatively important to cats by owners of cats in the study. These items included questions about their cat’s ability to walk, go up and down stairs, and eat among others. Using these items alone did not detect improvement or deterioration. The items that detected significant improvement at Day 36 following meloxicam treatment were 3, 5, 6, 7, 8, 9, 10, 12, 15, 17, 18, and 19. Items that detected significant deterioration following meloxicam administration at Day 57 were items 9, 10, and 11. However, the only items that were able to discriminate between meloxicam and placebo at Day 36 (greater improvement in the meloxicam group) were items 1 ($p = 0.019$), 12 ($p = 0.089$), and 14 ($p = 0.022$), while those able to discriminate between withdrawal of meloxicam and withdrawal of placebo at Day 57 (greater deterioration in the cats that had moved from meloxicam to placebo) were items 10 ($p = 0.018$), 11 ($p = 0.006$), 12 ($p = 0.030$), and 16 ($p = 0.060$).

Client Specific Outcome Measures (CSOM). For the parallel design, we first calculated Wilcoxon rank-sums tests for improvement of total CSOM scores on Days 36 (improvement) and 57 (deterioration). No statistically significant differences were found between the meloxicam group and the placebo group in this analysis. For the crossover design, we used a general

Table 6. Between group differences in change in activity counts over the first treatment period and subsequent masked washout period.

	Group	Mean (SD)	t-statistic	p-value
Improvement (Day 36 –Day 15)	PDPP	1.33 (6.86)	0.63	0.534
	PPPD	0.35 (4.88)		
Deterioration (Day 57 –Day 36)	PDPP	-4.25 (6.11)	-2.42	0.019
	PPPD	-0.37 (6.12)		

Data are averaged across days by cat.

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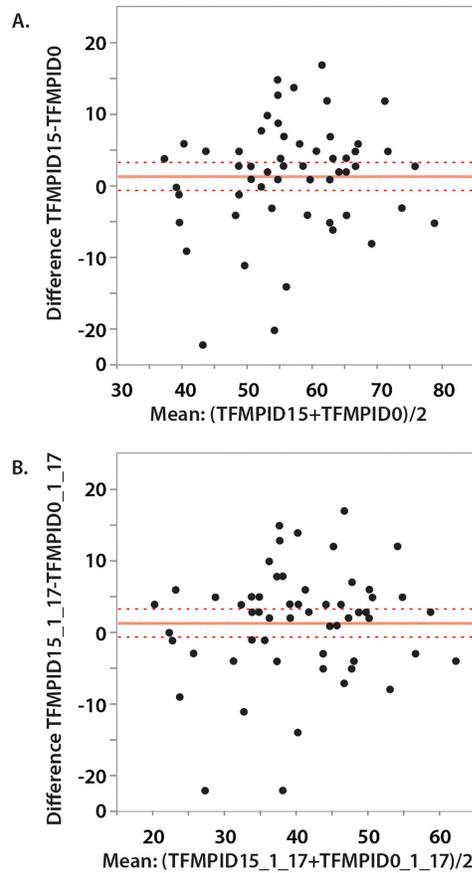


Fig 4. Bland-Altman plots of the FMPI for 17 items (A) and 19 items (B).

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linear model to analyze improvement in total CSOM scores and change in CSOM scores. In this model, there was a statistically significant time effect for change in total score (owners are more likely to report improvement at Day 36 than at Day 78) but no significant treatment effect (Table 9).

Quality of Life, Temperament, and Happiness Questionnaire. For the parallel design, we looked at owner reported changes in QoL, Temperament, and Happiness at Day 36 (vs. Day 15) and Day 57 (vs. Day 36) for improvement from baseline, and deterioration from Day 36 levels, respectively. No statistically significant changes were found (Table 10). The deterioration in Happiness was significant at the 10-percent level, more cats that had received meloxicam during Days 15–36 had a decrease in Happiness at Day 57 than the cats that had received placebo during Days 15–36.

Table 7. Summary of group comparisons of improvement / deterioration in the percent of possible points on the full FMPI questionnaire (Items 1–19), percent of possible points on Items 1–17 from the FMPI questionnaire, and improvement / deterioration in raw scores (Items 1–17) between Day 36 and Day 15 / between Day 36 and Day 57 respectively.

FMPI Q1-Q19: Percent Possible	PDPP		PPPD		T-test	
	Mean	Std.Dev.	Mean	Std.Dev.	T-statistic	p-value
Improvement, Day 36 –Day 15	0.107	0.106	0.100	0.100	0.26	0.794
Deterioration, Day 57 –Day 36	-0.056	0.121	-0.001	0.126	-1.69	0.097
FMPI Q1-Q17: Percent Possible						
Improvement, Day 36 –Day 15	0.102	0.105	0.092	0.101	0.38	0.706
Deterioration, Day 57 –Day 36	-0.055	0.113	0.006	0.118	-2.03	0.048
FMPI Q1-Q17: Raw Scores						
Improvement, Day 36 –Day 15	6.379	7.063	6.379	6.769	0.00	1.000
Deterioration, Day 57 –Day 36	-3.414	7.765	-0.276	7.695	-1.54	0.130

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When evaluating owner reported scores rather than owner reported change for QoL, there were no significant differences between groups for Day 36-Day 15 scores (improvement) or Day 57-Day 36 scores (deterioration).

For the crossover design, analysis of owner reported change in QoL, Temperament, and Happiness using a GEE analysis found a significant time effect for QoL ($p = 0.017$) such that owners were more likely to report improvement in QoL at Day 36 than at Day 78, regardless of treatment. No other significant effects were found for improvement.

Table 8. Summary of general linear model analysis of improvement in the percent of possible points on the FMPI questionnaire (Items 1–19 and 1–17) and improvement in raw scores (Items 1–17) on Day 36 and Day 78.

Improvement (Day 36 and Day 78)			
FMPI Q1-Q19; Improvement in Percent Possible			
Source	DF	F Value	Pr > F
Treatment	1	4.72	0.034
Time	1	1.01	0.318
Sequence	1	0.00	0.952
Error	54		
Corrected Total	111		
FMPI Q1-Q17; Improvement in Percent Possible			
Source	DF	F Value	Pr > F
Treatment	1	6.07	0.017
Time	1	1.09	0.301
Sequence	1	0.02	0.882
Error	54		
Corrected Total	111		
FMPI Q1-Q17; Improvement in Raw Scores			
Source	DF	F Value	Pr > F
Treatment	1	3.30	0.075
Time	1	0.58	0.451
Sequence	1	0.04	0.852
Error	54		
Corrected Total	111		

doi:10.1371/journal.pone.0131839.t008

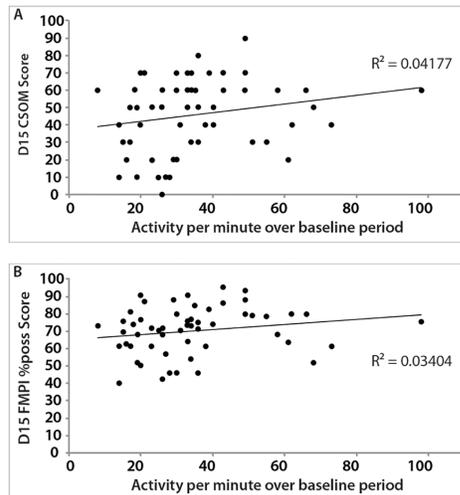


Fig 5. Relationship between average activity count and CSOM score at Day 15 (A) and FMPI %poss score at Day 15 (B).

doi:10.1371/journal.pone.0131839.g005

Discussion

With activity monitor counts used as our reference standard, we saw a significant treatment effect of low-dose oral meloxicam in increasing activity counts in cats with degenerative joint disease. Significantly higher levels of activity were detected in the meloxicam treated cats during the treatment periods. Inter-cat variability in activity counts were high. This is likely to be mainly due to differences in activity between cats, though reasons for these differences are unknown. Potential sources of variability include indoor vs. indoor/outdoor status, presence of stairs or size of home, and day of the week, as well as age, impairment, weight, and other factors. In this study, we restricted enrollment to cats that were indoors only, as indoor/outdoor cats have been shown to have different activity patterns, particularly if outside overnight.^[23] This also decreases the effect that outside weather would have on activity. All cats in the study had approximately the same number of week and weekend days included in the analysis, and

Table 9. Summary of general linear model analysis of improvement / deterioration in the total score across all three CSOM measures on Day 36 and Day 78 / on Day 57 and Day 99 respectively as well as change in total score.

	Source	D.F.	F-Value	P-value
Total Score, Day 36 and Day 78	Time (Day 36 and 78)	1	0.26	0.612
	Treatment (Meloxicam vs. Placebo)	1	0.62	0.433
	Sequence	1	0.37	0.544
Change in Total Score (Day 36 –Day 15, Day 78 –Day 57)	Time (Day 36 and 78)	1	23.09	<0.001
	Treatment (Meloxicam vs. Placebo)	1	0.06	0.814
	Sequence	1	0.03	0.862

Change was calculated as Day 36 –Day15, and Day 57 –Day 36.

doi:10.1371/journal.pone.0131839.t009

Table 10. Improvement in owner reported Change for Quality of Life, Temperament, and Happiness at Day 36 (from Day 15) and deterioration in owner reported Change for Quality of Life, Temperament, and Happiness at Day 57 (from Day 36).

Effect	Improvement at Day 36		Effect	Deterioration at Day 57	
	Chi-Square	p-value		Chi-Square	p-value
Quality of Life	1.02	0.313	Quality of Life	1.27	0.259
Temperament	0.67	0.412	Temperament	0.42	0.518
Happiness	0.09	0.764	Happiness	2.98	0.084

doi:10.1371/journal.pone.0131839.t010

other factors were shown to be similar between groups of cats, so while these might contribute to individual variability, they were evenly distributed between treatment groups. In addition, recent data from our laboratory (Hansen, unpublished data) indicates that the output from different accelerometers can vary significantly (although each is stable over time), suggesting caution be exercised when comparing data between accelerometers. Considering both these aspects, we therefore consider within group comparisons more appropriate. When evaluating intra-cat/intra-group changes we found a significant treatment effect where meloxicam increased activity counts in cats with DJD. Further, we saw a significant deterioration in activity counts during the period following withdrawal of active medication in the PDPP sequence group. The same analysis was not available for all cats in the PPPD group. Other studies using meloxicam in cats with DJD or osteoarthritis have found similar treatment effects with meloxicam on improvement of activity counts.[8] Two studies using client-owned cats found that daily administration of meloxicam led to increased daily activity counts. In these studies, cats were selected based on owner-noted mobility impairment, and veterinary diagnosis of degenerative joint disease, and cats were maintained in their home environment throughout the study. Using laboratory housed cats, Guillot et al found that night time activity counts were higher in normal (non-arthritic) cats than in OA cats,[24] and later found that night-time activity counts increased in cats on meloxicam at 0.025 mg/kg and 0.05 mg/kg daily, though not in the cats receiving meloxicam at 0.04 mg/kg/day.[25]

The current study detected differences between cats receiving meloxicam vs. placebo using subjective measures of owner report captured with the FMPI. The FMPI was developed using appropriate methods.[7–9] In previous work, the FMPI was shown to have discriminatory validity,[7] and good repeatability (test-re-test reliability).[7] However, in the present study using cats with higher impairment, repeatability was not as good. In the present study, although test-retest reliability of the FMPI instrument was reasonable, scores on Day 15 were significantly higher than on Day 0. Although the actual difference in number of points was small, this indicates that owners are rating their cats as significantly less impaired on Day 15 following a 2-week period of known placebo administration. We believe this justifies the use of the Day 15 scores as the baseline. The period from Day 0–Day 15 may serve as a learning period for the owners as well as a chance for owners to better observe the behavior of their cats. By requesting owners to give a daily medication (in the exact same manner as in the masked portion of the study) we likely changed the observation of cats by owners, and perhaps also altered the behavior of the cat, which might explain some of the differences in repeatability found in the present study. The present study also included a new analysis, the percent possible calculation for the FMPI, as well as enrolling more cats than the previous evaluation of the FMPI.

As has been described for dogs with osteoarthritis [26], we also observed a sizeable caregiver placebo effect in owner reports during the first treatment period. Using the analysis of the percent possible score for the 17-item FMPI (excluding the two VAS scale items), this instrument is able to detect improvement in the crossover design, but not in the parallel design due to the

caregiver placebo effect. This suggests that one way to adjust for the initial caregiver placebo effect is to use a crossover study design. Using the full cross-over design allowed for a difference between groups to be detected in objective actimetry during the second treatment phase. However, the full cross-over design creates a long, cumbersome clinical study. There are few masked, placebo controlled clinical studies assessing pain relief in client-owned cats with chronic pain [8, 15, 27] and large placebo effects have been seen in these studies as well. Although it has not been evaluated in detail, the placebo effect in these studies appears larger than is seen in comparable canine studies. This could be due to a lack of specificity in the CMI, or could be due to owners expectation of a positive effect combined with an overall lack of awareness of owners to their cat's behaviors. Study design may also be improved by better training of owners to recognize signs of pain in their cats, particularly those that are most responsive to analgesics.

The use of a percent possible score for the 17-item FMPI was also able to detect a deterioration following withdrawal of active medication that is not seen with the withdrawal of placebo. This effect can also be seen with a success/failure paradigm for the FMPI and CSOM (though selection of threshold for the FMPI may require further evaluation). [17] These data parallel the decrease in activity counts seen when active medication is withdrawn. Interestingly, though only significant at the 10% level, owner ratings of cat happiness also show the deterioration effect following the masked washout. Our measures of temperament and quality of life, and change in temperament and quality of life did not detect the presumed efficacy of meloxicam, despite previous work indicating strong relationships between the presence of DJD and both temperament [3, 8, 28] and quality of life. [7, 29]

Return of clinical signs following randomized withdrawal of active medication is a clinical trial design that has been used in humans, however, to our knowledge, all studies using this approach fall into the category 'Enriched Enrollment, Randomized Withdrawal' (EERW). [30–32] EERW designs select for treatment responders at the start of study, followed by randomization to continuation of the treatment or a switch to a placebo or active control to measure efficacy. These studies may then compare the treatment to the placebo/active control. One complaint about this design is the length of time required for the study. [32] Our study design did not select for treatment responders, and we refer to our design using randomized allocation and withdrawal as the 'RAW' design. We believe RAW designed clinical trials may be very useful in evaluating analgesics for chronic pain management in cats, both for placebo controlled as well as analgesic comparison studies. [33] While replication is necessary, if effective, this study design provides for shorter study duration, and less cumbersome clinical trials.

Owner ratings of item importance for the FMPI were not fully indicative of the items in which improvement or deterioration were seen, or those where a difference was detected between meloxicam and placebo. Future work will investigate whether owner ratings of importance can be used to give weight to individual FMPI questions for individual owners, or used to refine the structure of the FMPI. One important consideration when considering refinements or modifications to the FMPI is the effect of the order of the questions, or whether particular questions are important in encouraging respondents to reflect on their cat's behavior. The first step in selection of questions for an abbreviated FMPI would be to compare administration of the full instrument, with *a priori* decision to analyze only a subset of items, with a shortened form. Interestingly, while the FMPI, as percent possible, was able to detect a treatment effect, only one question, asking about the cat's ability to stretch, distinguished between meloxicam and placebo in both the treatment and deterioration phase, and this was only if one accepted a p-value at the 10% level for the treatment phase. However, there was no significant relationship between baseline scores of ability to stretch and baseline activity counts, despite the fact that AM data was also able to detect treatment effects and the effects of treatment withdrawal.

Previous studies with cats have looked at behavioral domains or specific behaviors that are responsive to analgesic treatment. A study by Clarke and Bennett showed owner ratings of improvement in willingness to jump, height of jump, and gait stiffness in cats with osteoarthritis that received meloxicam.[34] A second study by Bennett and Morton using meloxicam in cats with osteoarthritis found that owners rated positive changes in their cats in mobility, grooming habits, and temperament, with the greatest positive change in activity, though no placebo group was included for comparison.[28] A study by Klinck et al. used a survey to investigate, among others, additional signs for OA detection and monitoring in cats based on owners' observations.[3] This study found that owners reported changes in several specific behaviors, with jumping, stair use, and alterations in gait being present in $\geq 75\%$ of cats with OA. In addition, owners reported that jumping, stair use, speed, gait, mood, activity level, and daily schedule were perceived as responsive to treatment in $\geq 50\%$ of the OA-affected cats. Future work should consider gathering video data on cats in the home environment, before and after treatment, and creating detailed ethograms of what behaviors are performed and change, as well as owner input on the changes they detected. This information may then form the basis of developing the next generation of CMIs.

Our data indicates that the incorporation of the final two questions on the FMPI (asking about pain levels over the last 3 weeks, and pain levels today) did not improve the sensitivity of the FMPI, and our current recommendations are that these are dropped from the instrument. We believe these changes can be made without further evaluation because they were the last 2 questions on the FMPI and so deleting them will not influence how earlier questions are answered.

Additional objective measures could also be incorporated into the management of cats with DJD, as well as for further development and validation of CMIs. Several objective measurement systems have been tested in cats, including peak vertical ground force reaction [24, 25, 35] and goniometry.[6] In particular, peak vertical ground force reaction (PVG) has been shown to be moderately feasible in client-owned cats (8/23 cats refused to traverse the walkway),[35] and able to distinguish between normal cats and those with DJD [24], but was not able to show a treatment effect for meloxicam in cats with DJD.[25] This measure was not included in the present study, however, it is possible that complementary measures to actimetry would allow for increased sensitivity in the refinement of CMIs.

Finally, we have suggested that the data from Day 99 might not be as useful as data gathered earlier in the study. There are several reasons for this observation, including the smaller number of cats completing this time point. We found clinician impressions that owners were experiencing study fatigue, and were not as diligent in completing their surveys during this final evaluation. In addition, to remain in line with the earlier study protocol, the cats themselves had their final appointments on Day 78, and labwork/physical exam findings had already been discussed. It is our feeling that the final masked washout period may have occurred after the study had almost "ended" in the minds of the owners, despite the investigators informing them that a further treatment period, which could be active medication or placebo, occurred during days 78–99.

In this study, we used the NSAID meloxicam as our therapeutic intervention. The low-dose of meloxicam used was generally tolerated well by most cats, and can be regarded as efficacious in the management of DJD-associated chronic pain. This study enrolled cats with mild-moderate renal insufficiency and, as reported in Gowan,[14] overall changes in creatinine and blood urea nitrogen were not observed. While the cats in the PPPD group had a higher decrease in albumin from Day 0 to Day 78 than those in the PDPP group, this is of unknown clinical significance in the absence of other changes. The number of cats showing vomiting were fairly evenly distributed to active and placebo treatments. However, rates of adverse renal effects

including acute kidney injury were higher during or immediately following meloxicam administration than following placebo administration, warranting further investigation. In the most extreme case, a cat on meloxicam was vomiting for several days prior to the owner reporting this to investigators. It is not known, but is supposed, that earlier intervention would have mitigated the extent of this cat's kidney injury, as meloxicam is tolerated fairly well by cats with chronic kidney disease that maintain euvoolemia.[36] Given the therapeutic effectiveness of this medication, more sensitive early markers of injury, or screening tests able to predict tolerance of this class of drug would be of high value. This is especially important as recent work has shown that the overlap of DJD and CKD is great.[37]

Conclusions

In conclusion, when evaluating treatments for DJD-associated chronic pain, we recommend a masked, parallel study design, which incorporates a masked washout period to allow for the assessment of deterioration (RAW design), and use of actimetry as a reference standard. We recommend the use of actimetry only for within group paired comparisons, and comparisons between groups in the degree of change. Actimetry values are too variable from cat to cat to allow for direct between group comparisons. Additionally, we recommend the use of the 17-item FMPI, expressed as a percent of possible, to detect deterioration. Finally, we recommend the use of success/failure criteria for the FMPI to detect deterioration, or the use of success/failure criteria for the CSOM to detect deterioration.

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Supporting Information

S1 Appendix. Feline Musculoskeletal Pain Index. The FMPI is also available for download at: <http://www.cvm.ncsu.edu/docs/cprl/fmpi.html> as used for this study, and as the currently recommended 17-item version. (PDF)

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Author Contributions

Conceived and designed the experiments: MEG EHG AET BDXL. Performed the experiments: MEG AET WS. Analyzed the data: MEG EHG BDXL. Wrote the paper: MEG AET EHG BDXL. Identification of qualifying cats: WS.

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Supplemental Figure 1: Feline Musculoskeletal Pain Index



NAME:

DATE:

FELINE MUSCULOSKELETAL PAIN INDEX

Please take some time to complete the following questions.

Please mark the circle that best describes your cat’s ability to perform the following activities.

1. Walk and/or move easily?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

2. Run?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

3. Jump up (how well and how easily)?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

4. Jump up to kitchen-counter height in one try?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable



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NAME:

DATE:

Please rate your cat's ability to:

5. Jump down (how well and how easily)?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

6. Climb up stairs or steps?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

7. Go down stairs or steps?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

8. Play with toys and/or chase objects?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

9. Play and interact with other pets?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable



NAME:

DATE:

Please rate your cat's ability to:

10. Get up from a resting position?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

11. Lie and/or sit down?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

12. Stretch?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

13. Groom himself or herself?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

14. Interact with you and family members?					
<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable



NAME:

DATE:

Please rate your cat's ability to:

15. Tolerate being touched and/or held?					
○	○	○	○	○	○
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

16. Eat?					
○	○	○	○	○	○
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

17. Use the litter box (get in and out, squat, cover waste?)					
○	○	○	○	○	○
Normal	Not quite normal	Somewhat worse than normal	Barely, or with great effort	Not at all	Don't know or not applicable

How does your cat feel?

18. Please mark the point on the line that best describes your cat's pain over the past two weeks:

No pain Severe pain Don't know

19. Please mark the point on the line that best describes your cat's pain today:

No pain Severe pain Don't know

**Chapter 3c. Detection of Clinically Relevant Pain Relief in Cats with Degenerative
Joint Disease Associated Pain**

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Detection of Clinically Relevant Pain Relief in Cats with Degenerative Joint Disease Associated Pain

M.E. Gruen, E. Griffith, A. Thomson, W. Simpson, and B.D.X. Lascelles

Background: Detection of clinically relevant pain relief in cats with degenerative joint disease (DJD) is complicated by a lack of validated outcome measures and a placebo effect.

Hypothesis/Objectives: To evaluate a novel approach for detection of pain relief in cats with DJD.

Animals: Fifty-eight client-owned cats.

Methods: Prospective, double-masked, placebo-controlled, stratified, randomized, clinical study. Enrolled cats were 6–21 years of age, with owner-observed mobility impairment, evidence of pain in at least 2 joints during orthopedic examination, and overlapping radiographic evidence of DJD, and underwent a 2-week baseline period, 3-week treatment period with placebo or meloxicam, and 3-week masked washout period. Outcome measures were evaluated at days 0, 15, 36, and 57.

Results: Both groups significantly improved after the treatment period (day 36) on client-specific outcome measures (CSOM) and feline musculoskeletal pain index (FMPI) ($P < .0001$ for both); there was no difference between the groups on CSOM or FMPI score improvement. After the masked washout period, more cats that received meloxicam during the treatment period had a clinically relevant decrease in CSOM score ($P = .048$) and FMPI score ($P = .021$) than cats that received placebo.

Conclusions and Clinical Importance: Using both a client-specific and a general clinical metrology instrument, owners of cats with DJD were able to detect evident recurrence of clinical signs after withdrawal of active medication than after withdrawal of placebo, and that this study design might be a novel and useful way to circumvent the placebo effect and detect the efficacy of pain-relieving medications.

Key words: Client-specific outcome measures; Degenerative joint disease; Osteoarthritis.

Clinical responsiveness to an analgesic is typically measured by quantifying improvement during a treatment period, whether by self/proxy report or objective measurement. In noninferiority trials, efficacy of an analgesic is measured against a known effect whereas in placebo-controlled trials, typically considered the reference standard, efficacy above any placebo effect is required. In evaluating the efficacy of a pain-relieving medication in humans, questionnaire-based results are frequently used as people can simply be asked to rate their response to a given treatment. Despite the direct questioning of an individual's experience, a large placebo effect exists and is seen repeatedly in clinical trials.¹ In veterinary medicine, questionnaire-based studies involve owners as proxies, with owners and caregivers being asked to rate a response in their pet. The questionnaires employed may be general, as in the feline musculoskeletal pain index (FMPI),² inquiring about activities common to

Abbreviations:

CSOM	client-specific outcome measures
DJD	degenerative joint disease
FMPI	feline musculoskeletal pain index
OA	osteoarthritis

most cats, or they may be individualized, such as with the client-specific outcome measures (CSOM)² assessment. With both general and specific assessment techniques, large placebo effects occur in dogs³ and cats with DJD-associated pain.^{2,4}

Degenerative joint disease (DJD) is a frequent cause of pain in older cats. The prevalence of radiographic DJD in older cats is high, ranging from 22 to 90% of cats.⁵ Long-term treatment with nonsteroidal anti-inflammatory medications, useful in treating osteoarthritis (OA) in humans and dogs, has been suggested to be effective in cats in studies without a placebo control.⁵ However, when evaluated against a placebo in client-owned animals, the strong placebo effect obscures the presumed treatment-related effect.² This placebo response makes it difficult to demonstrate the efficacy of treatments to relieve pain in companion animal species.

In a novel clinical study design measuring responsiveness to a nonsteroidal anti-inflammatory medication, meloxicam,⁶ we have used change in pain status after cessation of treatment as a proxy measure of efficacy. We hypothesize that though efficacy over a placebo may be difficult to detect during a treatment period because of the placebo effect, owners of cats with DJD will notice recurrence of clinical signs after

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withdrawal of active medication compared to placebo, and that this may be a useful way to detect the utility of pain-relieving medications.

Materials and Methods

Cats were screened and enrolled similarly to previous studies conducted by the authors to assess joint pain in cats.² Sixty-six client-owned cats were identified by their caregivers or participating primary care veterinarians as study candidates based on owner-reported mobility impairment of at least 3 months duration and a history of veterinarian diagnosis or suspicion of DJD. Cats had to live indoors only, not be receiving any anti-inflammatory medication, and considered generally healthy before screening. At screening (day 0), cats received a physical, orthopedic, and neurologic exam, and blood and urine samples were taken for a hematology profile, serum biochemistry panel, urinalysis with sediment evaluation, and serum T4 analysis. Orthogonal radiographs of the complete axial and appendicular skeleton were completed under sedation and were reviewed by a board-certified veterinary radiologist. Also on day 0, owners completed a battery of subjective evaluations, including a CSOM questionnaire² (Fig 1) where owners selected 3 activities in which their cat was impaired and rated how much difficulty their cat had with each activity, and the FMPI.² Inclusion criteria included owner-perceived mobility impairment, evidence of pain in at least 2 joints during orthopedic examination and overlapping radiographic evidence of DJD, and absence of systemic illness. These criteria were more stringent (ie, resulted in recruitment of more highly impaired cats) than in previous studies.^{2,4} Cats with stable chronic kidney disease (up to IRIS stage 2) were eligible for participation. Qualifying cats were then enrolled into the study, and randomized (stratified by impairment) to receive meloxicam at 0.035 mg/kg/d or volume-matched placebo (identical to the vehicle for the active medication) during the treatment period. Meloxicam is approved in Europe and other countries for use in cats as a single subcutaneous injection and for repeated oral administration; however, in the United States it is only licensed for single subcutaneous injection. Stratification was done by owner-perceived impairment level and based on day 0 CSOM score to ensure that the cats with the highest degree of owner-perceived impairment were balanced in both treatment groups. No analysis based on level of impairment was performed.

After enrollment, cats began a 2-week baseline period during which they received volume-matched placebo (known to be placebo by both owners and investigators) in order to acclimate to the daily medication regimen, and to verify correct owner administration and record keeping. After this baseline, cats began one 3-week double-masked treatment period during which they received either meloxicam or placebo, and then a 3-week masked washout period (all cats received placebo, unbeknownst to the owners). The CSOM and FMPI were completed after baseline

(day 15) and after each 3-week treatment period (on day 36 and 57) (Fig 2).

Differences between the treatment groups were analyzed using *t*-tests for age and weight and chi-square analysis for sex. CSOM scores were calculated by assigning a score from 0 to 4 (0 = impossible, 4 = no problem) for owners' ranking of each of 3 activities to produce a score that could range from 0 (all activities were impossible for the cat) to 12 (all activities were no problem for the cat). A change in CSOM score greater than or equal to 2 (representing a 16.7% change in total score), as has been used in dogs,⁶ was considered relevant. To investigate improvement from day 15 to day 36, the number of cats in each group (meloxicam versus placebo) with CSOM changes greater than or equal to +2 were compared using one-tailed Fisher's exact test. Similarly for deterioration from day 37 through day 57, the number of cats in each group with CSOM changes less than or equal to -2 were compared using one-tailed Fisher's exact test. [Correction made after online publication February 10, 2014: "Fisher's exact test" has been updated to "one-tailed Fisher's exact test"]

Feline musculoskeletal pain index scores were analyzed in a similar manner. Scores from 1 to 5 were assigned for the owners' response to each questionnaire item (1 = not at all, 5 = normal). The total score for the 17 items could range from 17 (cats were unable to perform each item) to 85 (cats were normal for each item). A change in FMPI score of greater than or equal to +8 for improvement, or less than or equal to -8 for deterioration

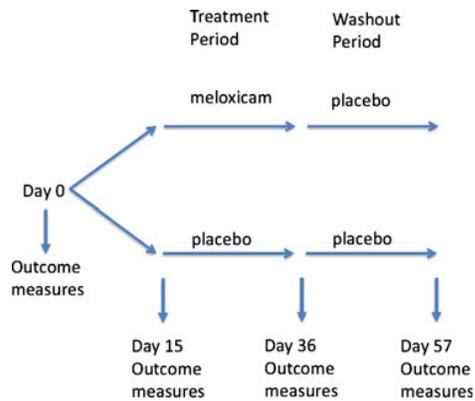


Fig 2. Study timeline. Outcome measures collected included client-specific outcome measures and feline musculoskeletal pain index.

Client Specific Outcome Measures

How much difficulty has your cat had over the last week for the following activities:

Activity	No Problem	Mild Difficulty	Moderate Difficulty	Severe Difficulty	Impossible	Compared to before starting medication 2 weeks ago, the ability is:
						<input type="checkbox"/> Worse <input type="checkbox"/> Same <input type="checkbox"/> Improved
						<input type="checkbox"/> Worse <input type="checkbox"/> Same <input type="checkbox"/> Improved
						<input type="checkbox"/> Worse <input type="checkbox"/> Same <input type="checkbox"/> Improved

Fig 1. Client-specific outcome measure form completed by owners of enrolled cats on days 15, 36, and 57.

(11.8% of total score range), was considered relevant. The number of cats with changes in FMPI score that were equal or greater in magnitude +8 for improvement or -8 for deterioration was compared using Fisher's exact tests. Changes from baseline (day 15) to day 36 for each group (CSOM and FMPI) were evaluated using *t*-tests. A *P*-value of >.05 was chosen for statistical significance. Data were analyzed using a statistical software package.^b

All procedures were approved by the North Carolina State University Animal Care and Use Committee before study initiation.

Results

Subjects

At enrollment, cats ranged in age from 6 to 21 years of age. There were no differences in age (*P* = .69), weight (*P* = .67), or sex (*P* = .79) between the placebo and meloxicam groups. A minority of cats in each group were rated as higher impairment (within our range of impairment) by their owners (based on day 0 CSOM scores) and were distributed evenly across both groups (n = 7 in the meloxicam group, n = 5 in the placebo group). Of the 66 cats enrolled in the study, 8 cats were excluded because of adverse events precluding the completion of day 57 (n = 5) and client non-compliance with the protocol (n = 3). A total of 58 cats (29 in each group) were included in the analysis of CSOM and FMPI improvement and FMPI deterioration, and 57 were included in the analysis of CSOM deterioration as one owner had failed to fully complete the CSOM questionnaire on day 57.

Improvement at Day 36 (from Day 15)

No significant difference was found between the placebo and meloxicam group in the number of cats with a change in CSOM score greater than or equal to +2

Table 1. Client-specific outcome measures (CSOM) improvement: number of cats with at least each amount of change in CSOM scores at day 36 (from day 15). Bold numbers indicate number of cats in each group at the chosen cut-off value.

	≥0	≥1	≥2	≥3	≥4	≥5
Placebo (n = 29)	27	24	19	15	9	5
Meloxicam (n = 29)	26	25	21	17	9	6

Table 2. Feline musculoskeletal pain index (FMPI) improvement: number of cats with at least each amount of change in FMPI scores at day 36 (from day 15). Bold numbers indicate number of cats in each group at the chosen cut-off value.

	≥0	≥1	≥2	≥3	≥4	≥5	≥6	≥7	≥8	≥9	≥10
Placebo (n = 29)	25	25	22	21	17	13	12	12	11	10	9
Meloxicam (n = 29)	24	23	22	19	18	17	16	15	11	9	8

(*P* = .39) or FMPI score greater than or equal to +8 (*P* = .61) (Tables 1, 2). Both groups improved significantly compared to baseline (day 15) on CSOM (*P* < .0001) and FMPI (*P* < .0001).

Deterioration at Day 57 (from Day 36)

A significant difference was found between the placebo and meloxicam group in the number of cats with a change in CSOM score greater than or equal to -2 (*P* = .048) and FMPI score greater than or equal to -8 (*P* = .021) (Tables 3 and 4).

Discussion

In this study, we used deterioration after withdrawal of active medication to show efficacy, above placebo, of a nonsteroidal anti-inflammatory medication for the treatment of DJD-associated pain in cats. The use of the masked washout period allowed us to detect a difference in behavioral ratings from owners on individualized (CSOM) and general (FMPI) subjective outcome measures. This allowed us to use a clinical phenomenon, the return of clinical signs after withdrawal of active medication, to circumvent the placebo effect that often complicates clinical trials of medications for pain relief, where improvement over placebo is the most common endpoint. As this placebo effect is seen in human as well as veterinary clinical trials, our finding might have translational relevance.

It is well established that DJD in cats leads to owner-perceived changes in behavior. Among the most commonly cited impairments include changes in a cat's ability to jump up or down, move smoothly up or down stairs, and ambulate normally. Changes are also seen in cats' activity level and mood. Several of these items have been shown to be responsive to treatment with analgesics,⁷ though the overwhelming placebo effect that may be seen makes interpretation of results difficult when evaluating analgesics in a clinical trial with client-owned cats.

We used an individualized measure to evaluate changes in cats' performance of activities in which they were impaired. These client-specific approaches have been found to be useful for veterinary and human pain research.^{8,9} We also employed a previously developed and evaluated questionnaire (FMPI) that queries owners on multiple items thought to be common among cats with DJD or mobility impairment.² In this study, responsiveness measured using the CSOM and FMPI at the end of the active treatment period was subject to the same placebo effect commonly seen in efficacy

Table 3. Client-specific outcome measures (CSOM) deterioration: number of cats with at least each amount of change in CSOM scores at day 57 (from day 36). Bold numbers indicate number of cats in each group at the chosen cut-off value.

	≤-5	≤-4	≤-3	≤-2	≤-1	≤0
Placebo (n = 28)	0	4	4	5	9	17
Meloxicam (n = 29)	1	4	9	12	17	21

Table 4. Feline musculoskeletal pain index (FMPI) deterioration: number of cats with at least each amount of change in FMPI scores at day 57 (from day 36). Bold numbers indicate number of cats in each group at the chosen cut-off value.

	≤-10	≤-9	≤-8	≤-7	≤-6	≤-5	≤-4	≤-3	≤-2	≤-1	≤0
Placebo (n = 29)	2	2	2	5	7	8	10	15	16	17	19
Meloxicam (n = 29)	5	7	9	10	10	10	12	13	15	17	19

trials for nonsteroidal anti-inflammatories⁷ and other analgesic medications, supplements, and diets.⁴ This placebo effect can mask findings of efficacy during a treatment period, as detecting improvement over this effect might be difficult.¹⁰ In addition, any natural waxing and waning of clinical signs, a feature of OA related pain, and the phenomenon of “regression to the mean,” might complicate the interpretation of repeated outcome measures. However, by comparing the ratings of owners during the masked washout period after a treatment period, we were able to show a greater change in ratings of the cats that had just received active medication versus those that received placebo. Our threshold for relevance of the change in CSOM scores was based on previous work in dogs⁶ where a change in 2 points (with 3 owner-identified activities) was considered clinically relevant. In cats, the CSOM has been used previously with a change in 4 points being considered clinically significant,⁸ however that study identified 5 activities with a total possible CSOM score of 20, whereas this study used only 3 activities with a total possible CSOM score of 12. Therefore, a change in magnitude of greater than or equal to 2 was considered a relevant change for this study, essentially converting the scores into a threshold for success versus failure of the treatment. For the FMPI, we selected a change in over 10% of the total possible score for relevance, but future studies will be needed to establish the best threshold for clinical relevance.

This study stratified cats by owner-perceived impairment based on day 0 CSOM scores. This was done to ensure that cats rated as highest impairment by their owners were equally distributed across the treatment groups. However, because of more stringent inclusion criteria based around disease burden and degree of mobility impairment, the cats in the present study were all more impaired than in previous studies we have conducted.^{2,4} Cats began the study at different points along the assessment scales, and these thresholds for improvement and deterioration were chosen for applicability across impairment levels. Other ways to stratify cats for impairment could include radiographic scoring of severity or baseline activity measured by actimetry, however it is still unknown how well these signs correlate with pain or with treatment response.

The effect of worsening clinical signs after withdrawal of medication is often discussed as a clinical phenomenon. This might be especially true for drugs used to treat chronic or progressive diseases. As clinicians, we might try a period of medication withdrawal, even if just while switching from one drug to another,

which might help both the owner and veterinarian to determine the efficacy of the medication. Despite its clinical use, this approach has not been used in drug trials in veterinary medicine (nor human medicine), though comments alluding to this phenomenon have been mentioned. In a nonplacebo-controlled study on the efficacy of meloxicam treatment for cats with OA, the authors highlight one particular cat in the discussion, in which the veterinary rating of improvement was greater than the owners’ rating on the questionnaire.⁵ They state that “when analgesic treatment was subsequently stopped, the owner soon became aware of a very obvious deterioration in the cat’s condition, bringing their retrospective assessment of improvement in line with that of the veterinary surgeon.”

It has been suggested¹⁰ that using multiple outcome measures may be more beneficial than using a single measure. The same authors noted that the placebo effect seen in ratings of behaviors in companion animals closely mimics what is seen in human clinical trials, making clinical trials for pain relief in veterinary medicine of important comparative value as well as veterinary value. It is our belief that study designs that incorporate a masked washout period and measure deterioration after this washout period may prove to be a better design for the detection of treatment effects over placebo effects. In this study, owners were masked during the washout period, whereas the investigators were not, providing the potential for an investigator to bias the owner ratings. However, the interviews with owners were conducted by trained investigators, in the same manner at each visit, thus limiting the likelihood that owners would be influenced in their ratings. In addition, investigators were masked to the treatment the cat had received during the treatment period. In the future, a double-masked washout period would increase the robustness of the findings by avoiding any potential for investigators to bias owner ratings. While this is a limited study, we believe that study designs that allow for analysis of this deterioration effect in addition to other efficacy measures may lead to breakthroughs in treatment options for chronic pain in our patients.

Footnotes

- ^a Metacam® 0.5 mg/mL Oral Suspension; Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO
^b JMP Pro 9.0.0; SAS, Cary, NC

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Conflict of Interest Declaration: Dr BDX Lascelles has received consulting and speaker fees from Boehringer Ingelheim Vetmedica, Inc.

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Chapter 4 : Objective measures

In this chapter, we turn our attention to objective assessments of pain and associated clinical signs in cats with DJD. The first part of this chapter describes the application of functional data analysis (FDA) to accelerometer data from cats. FDA allows for the study of patterns of activity in cats, a granularity of detail that has been lost in previous analyses. The second and third parts of this chapter detail discovery phase work on soluble biomarkers for pain and disease in DJD. We began with an evaluation of the agreement between serum and plasma concentrations of cytokines measured using multiplex technology in a small number of samples. We followed this with a larger study, using serum samples from 186 well-phenotyped cats and were able to assess relationships between radiographic DJD scores, orthopedic exam pain scores, and measured concentrations of 19 cytokines/chemokines.

**Chapter 4a. – The Use of Functional Data Analysis to Evaluate Activity in Cats with
and without Degenerative Joint Disease Associated Pain**

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To be submitted to PLOS Biology

Introduction

Physical activity is commonly affected in patients with degenerative joint diseases (DJD), and changes in activity may be used as an outcome measure for patients with arthritis and DJDs¹⁻⁴ Self-reported activity is often inadequate for quantifying actual physical activity,⁵ as self-report is subject to biases due to recall and social desirability, and are likely to under- or over-estimate true activity. The advent of accelerometer based systems allows for objective assessment of activity, and these systems have allowed researchers to more specifically define activity patterns, as well as track changes in activity related to health statuses and response to interventions.

‘Activity’ monitors (accelerometers) measure changes in acceleration by detecting low-frequency accelerations sampled at frequencies that vary by device but are typically between 1- and 15-seconds. These changes in acceleration are recorded by the device and are converted to ‘counts’ for a given epoch length. The counts are unit-less, and are generated by a voltage signal which is proportional to the individual unit’s measure (e.g. duration and/or intensity) of change in acceleration.⁶ That is, these counts will be higher with higher magnitude of acceleration for a given epoch. Accelerometers may be classified by the number of axes in which they measure acceleration (uni-, bi-, or tri-axial), or may be omnidirectional. These monitors have been evaluated in humans as measures of physical activity by comparison of activity counts against oxygen consumption, measured via indirect calorimetry,⁷ or doubly-labeled water.

There has been great interest in the ability to objectively measure physical activity since correlation coefficients between self-report and accelerometer-based measures are frequently low.⁸ In humans, accelerometer-based activity monitoring has been used to assess activity during sleep,⁹ following stroke,¹⁰ and as a criterion for validation of physical activity survey assessment tools.¹¹ Population based epidemiologic studies have used accelerometry to evaluate physical activity in people with arthritis^{3,12-14} while intervention studies have quantified effects of treatments on activity with some showing improvement¹⁵ and others showing more equivocal effects.¹⁶ Analytical methods across studies have varied resulting in calls for greater uniformity in the interpretation of accelerometer-based data.^{17,18}

The establishment of criteria for defining sedentary behavior and high-activity behavior has not been standardized. Activity counts per minute have been used to determine whether an individual is moving or sedentary, and sustained high levels of activity counts used to indicate high-intensity activity, but different studies have employed cut-off levels that lead to disparate conclusions. The effects of varied cut-offs were examined in a modeling experiment done by Masse et al.¹⁸ where application of four algorithms for accelerometer-based data reduction resulted in marked differences in outcomes. Additional issues include the mismatches between the detection abilities of particular activity monitors in relation to the activity being studied (e.g. uni-axial, vertically sensitive accelerometers used to detect activity involved in riding a bicycle),¹⁹ differences in output when worn on the hip vs. the wrist,²⁰ and varying criteria for establishing length of wear. Frequently algorithm based or visual inspection of data is used to determine estimated wear time. Despite these limitations,

accelerometry remains the primary means of objective physical activity monitoring in patient populations.

In veterinary medicine, accelerometers have been used in studies in many species. In dogs²¹ and in cats,²² accelerometry, with specific accelerometer types, has been validated in a lab environment as a surrogate measure of distance moved. In both dogs and cats, accelerometer output (counts) was compared against objectively assessed distance moved using standardized software (Noldus® Ethovision) designed for quantification of behavior.^{21,22} Validation studies were followed with feasibility studies to evaluate the ability of dogs and cats to tolerate wearing such monitors in their home environments. Interestingly, a study in dogs found that activity counts were higher on weekends as opposed to weekday,²³ while a study in cats found the opposite.²² In the cat study, the subjects were laboratory-housed cats, and the lower activity counts seen during the weekend were attributed to the lower amount of human/caretaker activity in the facility during non-weekdays. This suggests that owner patterns of activity are likely important mediators of activity in pet dogs and cats, and that activity patterns may not be uniform across the week.

The most common application of accelerometry in veterinary medicine has been the study of spontaneous activity in dogs and cats with DJD/osteoarthritis (DJD/OA). Several studies have used accelerometers to measure activity in dogs^{21,24} and cats.²⁵⁻²⁷ Moreover, both dogs²⁸ and cats with DJD/OA will show improvements in activity with analgesics, and motor activity has been used as an important objective outcome measure for analgesic treatments in cats with DJD/OA in multiple studies.^{25,29-32}

In addition, there is recent increased interest in domestic dogs and cats as models of naturally-occurring DJDs³³⁻³⁵ in humans, as both species develop spontaneous disease with significant overlapping features with the human condition.³⁶⁻³⁸ This includes owner-evaluated activity and mobility impairment. Indoor cats, in particular, are intriguing as a model of spontaneous activity, as their daily activity is less confounded by human intervention (i.e. though influenced by human activity, their activity over the day is not dependent on whether or not they are taken for a walk). Thus far, the analysis of the activity data generated by accelerometers in cats has been fairly coarse and a better understanding of activity patterns or profiles, and the most useful approaches for analyzing activity data will benefit both our ability to interpret the effects of DJD/OA on activity in cats, and the applicability of this naturally-occurring model to translational research.

To date, accelerometry has been used in cats to describe normal activity under different feeding and housing conditions³⁹⁻⁴¹ and activity in response to weight management strategies,^{42,43} in addition to the studies of analgesic treatments for DJD/OA and associated pain.²⁹⁻³² Across these studies, statistical analyses of activity data have varied widely in method, using a diversity of analytic designs, generally based upon condensed data. Particularly in the drug intervention studies, cats may wear activity monitors for days to weeks. Considering that each 24-hour day may contain 1440 individual per-minute “counts,” these studies generate large volumes of data for analysis. Current analytic methods frequently collapse the data down to single summary values (e.g. total counts or average per-minute counts) for particular time spans, and information about the pattern of activity in cats is lost. Inter-cat variability in these summary measures, even within a housing condition, is high,

making between group analyses difficult. This variability, coupled with a lack of knowledge of the most important metric to investigate, hinders the ability to fully understand the impact of disease and the effects of interventions on cat activity.

Functional data analysis (FDA) provides methods for analyzing data that is believed to arise from curves evaluated at a finite grid of points.⁴⁴ In particular, it allows for the use of the entire profile of daily activity counts (over a 24-hour day), rather than summary values. As a result, FDA allows analysis of data patterns without losing the richness of the information contained in the minute-by-minute counts.⁴⁵ A common technique in FDA is functional principal components analysis (FPCA), which examines the dominant modes of variation of the data as a method of understanding the major sources of data variability.⁴⁵ Functional data analysis has been applied to accelerometer data in recent studies of people,⁴⁶⁻⁴⁹ but to our knowledge has not been applied in the field of pain research or with data gathered from veterinary species.

Application of FDA to accelerometer data from cats offers an opportunity to examine the pattern of activity in cats including potential identification of peaks and quantification of the variability of activity, and the effects of covariates that vary over time.⁴⁸ FDA allows activity data from cats to be represented in new ways than have previously been described and can aid in the detection of patterns or variations among the data⁴⁵ as well as inform decisions about the use of such data in evaluating therapeutics.

The objectives of this study were to use FDA to evaluate activity patterns and activity intensity in cats with and without DJD in order to better understand normal population distributions for each group. We hypothesized that 1) normal cats would have higher daily activity counts and intensity than cats with DJD; 2) daily activity counts and intensity in cats with DJD would be inversely correlated with total radiographic DJD burden and total orthopedic pain score; and 3) daily activity counts and intensity would have a different pattern on weekends vs. weekdays. To our knowledge, no studies have been published that examine activity patterns in well phenotyped cats (with and without DJD) in their home setting.

Materials and Methods

Subjects

Potential study subjects were identified from local primary care veterinarians or were self-referred by owners in response to advertisements for one of four clinical trials. The first trial was designed to investigate activity in normal cats (i.e. those without DJD) (unpublished data). Two other trials included in the exploratory analyses^{25,30} were designed to evaluate outcome measures and efficacy for a non-steroidal anti-inflammatory medication in cats with DJD and owner-rated mobility impairment. Baseline data from cats enrolled in a fourth clinical trial designed to evaluate the efficacy of a biologic for DJD-associated pain (unpublished data) were used for confirmatory analyses.

Inclusion and Exclusion Criteria

Inclusion criteria for the normal cat study included age over 1 year, weight over 1 kg, and the absence of owner-rated mobility impairment. Inclusion criteria for the intervention trials have been previously described.^{25,30} Briefly, cats were required to be greater than 1 year of age and weigh more than 1 kg, and to have a qualifying degree of owner-rated mobility impairment, joint pain on orthopedic examination, and radiographic evidence of DJD.

Exclusion criteria for all trials was described previously^{25,30} and included the presence of suspected or diagnosed infectious diseases, symptomatic cardiac disease, immune-mediated disease, neoplasia, inflammatory bowel disease, urinary tract infection, hyperthyroidism, and diabetes mellitus. Cats with stable chronic kidney disease (CKD) up to and including IRIS stage two⁵⁰ were eligible to enroll following demonstration of stable serum biochemistry and urinalysis results. Importantly, all cats were required to be indoor only and able to wear a collar, though they did not need to have a collar at the time of enrollment.

Recruited cats were examined by a veterinarian and received full physical, orthopedic, and neurologic examinations. Demographic data including age, weight (kg), and body condition score ([BCS] on a 9-point scale⁵¹) were recorded. Cats meeting eligibility criteria were then sedated and orthogonal radiographs were taken of each joint. Radiographs were reviewed for the presence of DJD/OA as described in⁵² by a board-certified veterinary radiologist masked to the results of the orthopedic examination.

Total Pain Scores

During the orthopedic examination, each joint and axial skeletal segment was palpated and manipulated to evaluate for signs of pain and instability. Responses for each joint or segment were scored using a previously published scale⁵²: 0 = no resentment; 1 = mild withdrawal, mild resistance to manipulation; 2 = moderate withdrawal, body tenses, may orient to site, may vocalize/increase vocalization; 3 = orients to site, forcible withdrawal from manipulation, may vocalize or hiss or bite; 4 = tries to escape or prevent manipulation, bites or hisses, marked guarding of area. The scores for each individual joint or axial skeletal segment were summed to generate a total pain (TPain) score for each cat (possible range: 0-80). Scores from a previously described study of the prevalence of DJD in cats⁵² were used to develop categorical labels for TPain scores based on quartile distribution from the randomly selected population of cats that were evenly distributed across 4 age groups. Based on these classifications, TPain scores were further categorized as 0-2=negligible/normal (as long as no single joint received a score of 2); 2-4 = low (a score of 2 was placed in this category if a single joint received a score of 2); 5-9 = moderate; 10 and above = high.

Total DJD scores

Radiographs were evaluated and scored as previously described.⁵² Briefly, each joint was evaluated for the presence and severity of radiographic changes indicative of DJD and scored on a scale from 0 (normal) to 10 (ankylosis) by a single investigator (BDXL). The scores for each individual joint or axial skeletal segment were summed to generate a Total DJD (TDJD) score for each cat (possible range: 0-200). Again based on results of quartile

distributions from the randomly selected population of cats,⁵² DJD scores were further categorized as 0-3 = negligible/normal; 4-12 = low; 13-24 = moderate; 25 and above = high.

Activity monitors

Following enrollment, all cats in each study were fitted with an activity monitor (Actical®, Philips Respironics, Bend, Oregon, USA) mounted on a neck collar (Figure 1). Collars were provided by the study if the cats did not have their own. The Actical® monitors are omnidirectional activity monitors that contain a piezoelectric sensor mounted to an internal circuit board to generate analog voltage change that is proportional to the duration and intensity of the change in acceleration.⁶ Epoch length for summary data output by the unit was set to 1 minute, and the collars were worn continuously throughout the study period, with the exception of periodic downloading. Each daily activity profile is thus composed of 1440 minute-by-minute measurements. Activity data were downloaded to a dedicated computer via a serial port reader (Actireader®) using designated software. The software generates a graphical representation of the activity over each day (Figure 2) as well as a ‘raw’ output of activity counts per minute that can be exported into a spreadsheet for analysis. Cats in the normal activity group wore their collars for 15 days, and had 13 days of useable data (the initial and final day were deleted as cats had a variable number of hours with the collar on for these two days). Cats in the intervention studies wore their collars throughout their studies, and had 13 comparable baseline days (deleting the first day and the day the collars were brought in for download). Cats in the confirmatory analysis group had 13 days of useable baseline data.



Figure 1. Client-owned cat wearing a collar-mounted Actical® accelerometer in the typical position on the neck.

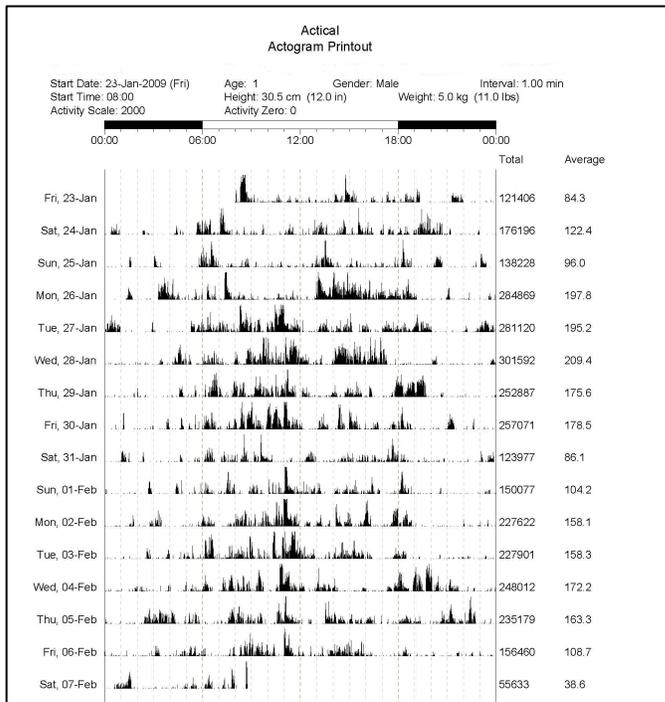


Figure 2. Example of an actigram for a cat. Each row of data contains the activity counts for a single day, with the counts depicted graphically along the time axis from 12:00 am to 11:59pm. The final two columns for each row represent the total activity counts for the day, and the average per-minute counts for the day.

Statistical analysis

Data disclosure

Portions of the data used for this manuscript have been previously published, however the current use and analysis represent original work. The activity data for the cats with DJD have appeared in two separate publications.^{25,30} The remaining data have not been previously published. Cats used for the confirmatory analysis (n = 33) were also part of an intervention study, had a comparable 13 days of baseline data as the cats in the DJD group, and only these baseline data are used here.

Data sets

The normal cat data set included cats that had no owner-noted mobility impairment, and normal classifications for TDJD score and TPain score and comprised data from n = 15 cats. Cats in the DJD data set had a combination of owner-noted mobility impairment and abnormal TDJD and TPain scores (n = 83). These cats represent populations from two individual studies referred to by their in-house names as FMPI (n = 25) and Low-Dose (n = 58). Cats in the confirmatory analysis (n = 33) also had a combination of owner-noted mobility impairment and abnormal TDJD and TPain scores.

Data analysis

Prior to the current FDA-based approaches, descriptive statistics were generated for demographic information for the cats and compared using one-way ANOVA for continuous variables (age, BCS, TPain score and TDJD score) and Chi-squared testing for distribution of cat gender.

As activity counts were highly skewed, data were transformed using the equation $x \rightarrow \ln(1 + x)$ and then averaged in each 5-minute interval to decrease variability.⁴⁶ For convenience, in the remainder of the paper we refer to the transformed data over the 5-minute intervals as activity counts. For all analyses, significance level was set at $\alpha=0.05$; when multiple tests were performed on the same data subset, a Bonferroni correction was applied as $\alpha = 0.05/(2*k)$ where k is the number of tests and the adjusted p-value reported. Average activity was calculated for each group of cats and denoted as $Y_{Gi}(t)$ for the activity of cat i in group G . Further, a measure of intensity of activity was generated to control for a cat- or accelerometer-specific effect and referred to as $I_{Gi}(t)$, for the intensity of activity of cat i in group G . Intensity provides a sense of the cat's activity relative to its average activity, with higher intensity interpreted as more active than average. It was calculated using a cat's average activity over the baseline period with the following equation:

$$I_{Gi}(t) = Y_{Gi}(t) - Ave_t Y_{Gi}(t)$$

Evaluation and comparison of activity profiles for normal cats and cats with DJD

To characterize activity patterns in cats with and without DJD, average activity and intensity profiles were separated for weekends (Saturday and Sunday) and weekdays (Monday through Friday) within each group of cats. Likelihood ratio testing⁵³ was used to formally assess first whether weekend and weekday activity and intensity profiles were different for each group of cats, and second whether the activity and intensity profiles were different for the two groups of DJD cats (FMPI and Low-dose). For both the activity and intensity profiles of the two groups of DJD cats, the null hypothesis of no difference between the two groups' means for weekends and weekdays was formally investigated in four main

settings: (1) no covariates; (2) controlling for age, BCS, and their interaction (age*BCS); (3) controlling for age, BCS, age*BCS interaction, and TPain score; (4) controlling for age, BCS, age*BCS interaction, and TDJD score. Null distributions were based on N=10,000 simulations. Further, we formally assessed whether the population distributions of activity and intensity profiles for the two groups of DJD cats were the same using the Anderson-Darling testing procedure proposed by Pomann et al.⁵⁴ Results are discussed in the results section; they were supportive of separating weekend and weekday activity and pooling data from the two groups of cats with DJD into one DJD group.

To describe the main features of the average activity and intensity profiles of the cats, functional principal components analysis (FPCA) was performed for both the Normal cats and the combined group of cats with DJD (DJD group), separately for weekends and weekdays. To understand the relationship between weekends and weekdays for each component, correlations were generated on the scores for each principal component. To formally assess the effects of age, BCS, TDJD score, and TPain score on activity profiles and intensity profiles, we used functional regression models for the Normal and DJD groups. Specifically, the assumed models for each response (and separately for weekends and weekdays) can be written for each group as specified in Faraway⁵⁵ and computed using Scheipl et al.⁵⁶ methods:

Model 1: Normal cats (where TDJD and TPain scores were not included)

$$Y_i(t) = \beta_0(t) + Age_i\beta_1(t) + BCS_i\beta_2(t) + (Age * BCS)_i\beta_3(t) + \epsilon_i(t)$$

Model 2: DJD cats

$$Y_i(t) = \beta_0(t) + Age_i\beta_1(t) + BCS_i\beta_2(t) + (Age * BCS)_i\beta_3(t) + TDJD_i\beta_4(t) + TPain_i\beta_5(t) + \epsilon_i(t)$$

where $\beta_0(t)$ is the intercept, $\beta_1(t)$ is the time-varying effect of age, $\beta_2(t)$ is the time-varying effects of BCS, $\beta_3(t)$ is the time-varying effect of the interaction between age and BCS, $\beta_4(t)$ is the time-varying effect of DJD score and $\beta_5(t)$ is the time varying effect of Pain score. Here $\epsilon_i(t)$ denotes the zero-mean residual term.

Finally, we compared the Normal group with the DJD group using both the daily activity and intensity profiles, separately for weekends and weekdays. Average activity profiles and intensity profiles for Normal cats and those with DJD were compared using the same analysis approach outlined for comparing the two groups of DJD cats for both group means and population distributions. Group means were formally investigated in the same four main settings, with and without covariates.

Confirmatory analyses

FPCA was performed using data from the cats in the confirmatory group. Results were compared visually with the results from the cats in the DJD group for pattern similarity, but were not formally tested against data from the DJD group due to the smaller sample size.

Results

Descriptive statistics for the cats in each group are presented in Table 1. Cats in the Normal group were significantly younger, and, as expected, had lower TDJD and TP scores than cats in the FMPI and Low-dose groups. Cats in the FMPI and Low-dose groups were not significantly different for any of these variables. Cats in the confirmatory group had significantly higher TPain scores than cats in the FMPI and Normal Cat groups.

Table 1. Demographic distribution for cats included in each of the studies. Results within a category that are designated by the same letter were not significantly different from one another.

Study	Age (mean years)	Body Condition Score (1-9) <i>median</i>	Sex (MC/FS)	Mean TDJD score (possible range:0-200)	Mean TPain score (possible range:0-80)
Normal cat (n=15)	5.80 - A	5	9/6	3.07 - A	1.3 - A
FMPI (n=25)	11.77 - B	7	8/17	20.0 - B	14.0 - B
Low-dose (n=58)	12.4 - B	6	27/31	23.4 - B	16.4 - B,C
Confirmatory (n=33)	12.2 - B	6	14/19	21.7 -B	18.9 - C
Between group analysis	ANOVA: p<0.001	Wilcoxon test: p=0.060	Likelihood ratio: p=0.360	ANOVA: p<0.0001	ANOVA: p<0.0001

Prior to transformation, range, quartiles, mean, and median average per minute activity were calculated (Table 2). The range of activity counts for the normal cats is smaller than for the cats with DJD, however the mean average per minute activity for each group is not significantly different (One way ANOVA, $p = 0.541$).

Table 2. Pre-transformation range, quartiles, median, and mean of average per-minute activity counts across the period for each group of cats.

Group	Minimum	1 st Quartile	Median	Mean	3 rd Quartile	Maximum
Normal	20.61	25.02	31.38	35.98	42.12	72.04
FMPI	11.44	22.09	30.87	39.70	54.92	105.70
Low-dose	8.22	21.31	31.42	34.97	41.05	108.00
NV-02	7.20	21.16	27.74	31.58	36.62	75.85

Evaluation of activity profiles for Normal cats

Figure 3 depicts the average activity and intensity profiles for weekdays and weekends for the Normal cats. The times where the cat's intensity is positive may be interpreted as times when the cat's activity is greater than their typical activity. Cats show a bimodal pattern of activity with a trough during the hours between 2 am and 5 am. During the weekdays, the activity peaks occur in the morning between 5:30 am – 9 am and in the evening between 5 pm – 11 pm. This pattern is present but less well-defined on weekends, with the morning peak less extreme and shifted to the right. Likelihood ratio testing for activity and intensity tested the null hypothesis that mean weekend and weekday average activity and intensity were the same and obtained p-values of <0.0001 for each, indicating a statistically significant difference between weekends and weekdays for means of average activity and intensity profiles.

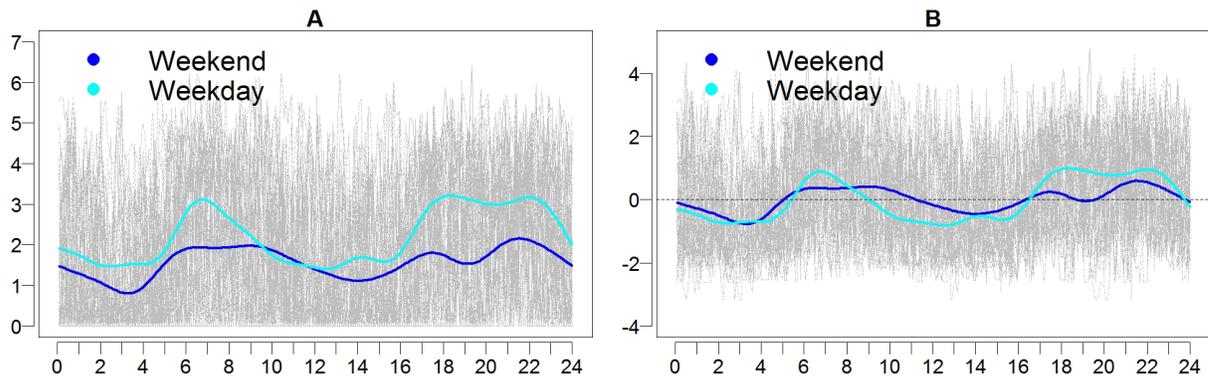


Figure 3. Activity (A) and intensity (B) profiles for cats in the Normal group. Log transformed activity for all cats is shown in gray with time (in hours) along the horizontal axis. The group mean for activity and intensity are shown for the weekends (dark blue) and weekdays (light blue). For intensity, positive values (above the zero line) indicate activity that is higher than average for that time period, while negative values (below the zero line) indicate activity that is lower than average.

Evaluation of activity profiles for DJD cats

The two sets of data from cats with DJD (FMPI and Low-dose studies) were evaluated for a difference in activity and intensity profiles prior to pooling. The test for a difference in the distributions showed no significant difference for the weekends ($p=0.114$) or the weekdays ($p=0.139$). Activity and intensity profiles from the two groups are shown in Figure 4. Results of likelihood ratio tests showed no significant differences between the two sets of cats in models that included no covariates ($p=0.790$ for activity and $p=0.986$ for intensity), or controlled for age, BCS, the interaction between age and BCS, TPain score, and TDJD score (all p -values >0.050 for both activity and intensity). Given the lack of evidence of a difference between the groups for activity and intensity profiles, the two groups were pooled.

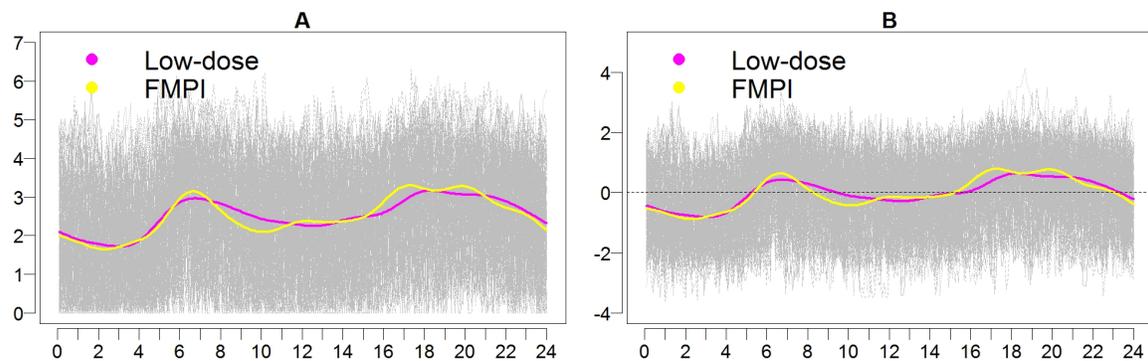


Figure 4. Activity (A) and intensity (B) profiles for cats in the two DJD groups. Log transformed activity for all cats is shown in gray with time (in hours) along the horizontal axis. The group mean for activity and intensity are shown for the Low-dose study (red) and FMPI study (yellow).

Following pooling of the data, and as with the Normal group of cats, average activity and intensity profiles for DJD cats for weekends and weekdays were generated (Figure 5).

Again, the bimodal pattern of activity was noted, with peaks in activity and intensity evident in the morning (5am – 8:30am) and evening (4pm – 11pm), particularly during weekdays and to a lesser extent during the weekends. Likelihood ratio tests for activity and intensity tested the null hypothesis that mean weekend and weekday average activity and intensity were the same and obtained p-values of <0.001 for each, indicating a difference between weekends and weekdays for means of average activity and intensity.

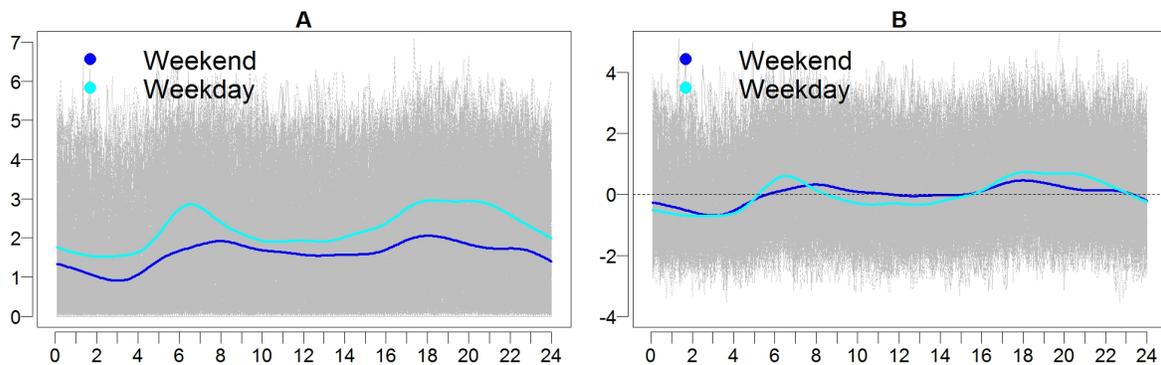


Figure 5. Activity (A) and intensity (B) profiles for cats in the DJD group. Log transformed activity for all cats is shown in gray with time (in hours) along the horizontal axis. The group mean for activity and intensity are shown for weekends (dark blue) and weekdays (light blue).

As weekend and weekday profiles have different distributions, FPCA was performed separately for weekend and weekday data, and results are presented here for intensity profiles for the Normal group, DJD group, and confirmatory group. The top three eigenfunctions are shown in Supplementary Tables 1-3. Figures 6-8 display the variation about the mean corresponding to each direction: $\hat{\mu}(t) \pm 2\sqrt{\hat{\lambda}_k\hat{\phi}_k}(t)$ for each top three eigenfunctions for the Normal group, DJD group, and confirmatory group, respectively. For the Normal group, the three components explain ~77% and ~86% of the total variance for weekend and weekday

intensity, respectively. Correlations between the scores for weekends and weekdays were 0.77 for FPC1, 0.53 for FPC2, and 0.64 for FPC3. For both weekends and weekdays, the variance about the mean for FPC1 shows a sign change at the beginning and end of each peak, while FPC3 shows an important peak during the evening on the weekdays that is shifted earlier on weekends. Cats that are positively loaded on FPC1 show similar behavior on weekends and weekdays during the period from midnight to 6am, but different behavior on weekends and weekdays during the period from noon to midnight.

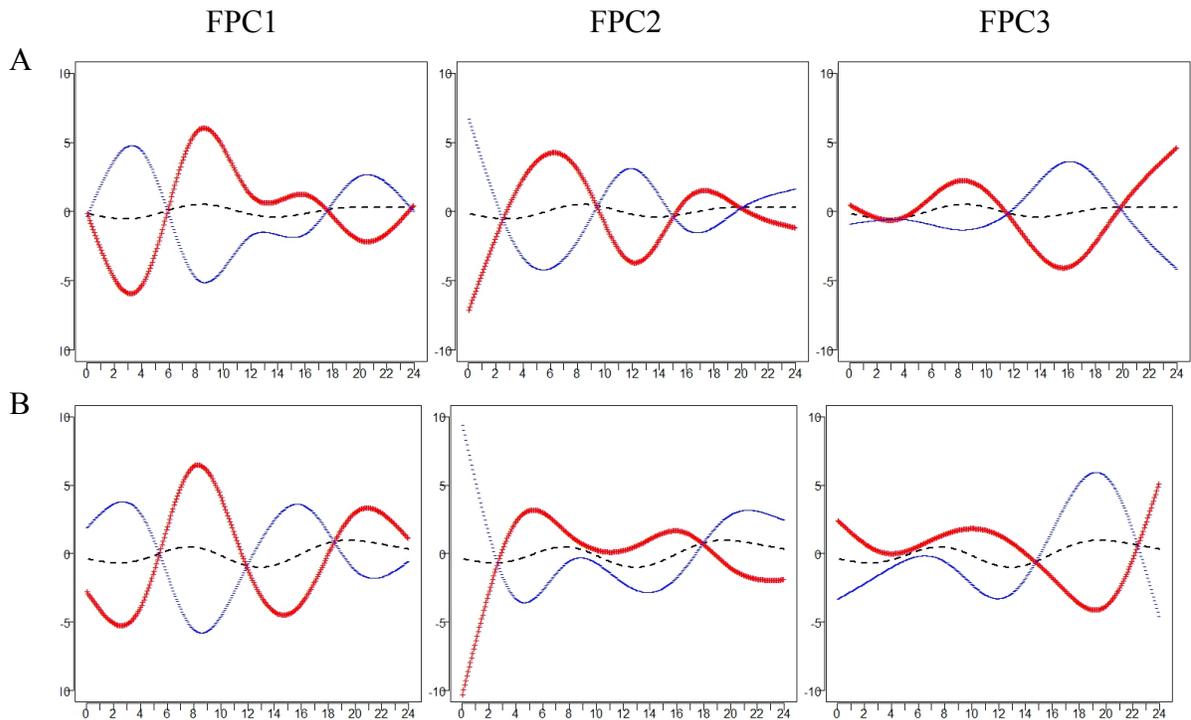


Figure 6. FPCA for Intensity for the Normal cats. Hours of the day are shown across the horizontal axis. Variance about the mean that corresponds to each FPC is shown for weekends (A) and weekdays (B) with red (pluses) indicating the positive direction and blue (minuses) indicating the negative direction. Variance explained by each FPC: Weekends: 35.88%, 24.44%, 16.83%. Weekdays: 40.01%, 23.86%, 21.77%.

For the DJD group, the three components explain $\sim 73\%$ and $\sim 77\%$ of the total variance for weekend and weekday intensity, respectively. Correlations between the scores for weekends and weekdays were 0.38 for FPC1, 0.41 for FPC2, and 0.49 for FPC3. For both weekends and weekdays, the variance about the mean for FPC1 shows a sign change at the beginning and end of each peak. Cats that are positively loaded on FPC1 have lower than average activity during early mornings on weekdays, higher than average activity between 10am and 3pm, and lower again after 3pm, while a different pattern is seen for weekends. FPC2 and FPC3 also show different patterns for weekends and weekdays, particularly between 3 pm and midnight for PFC2 and midnight to 8 am for FPC3.

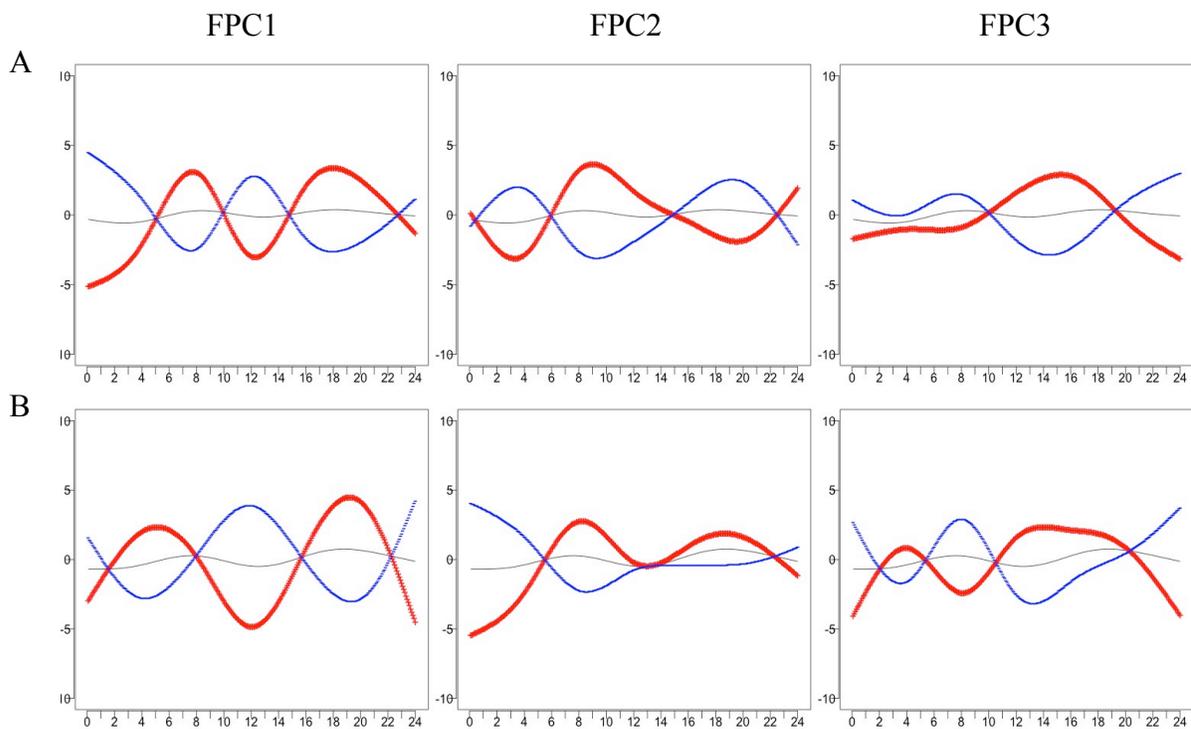


Figure 7. FPCA for Intensity for the DJD cats. Variance about the mean corresponding to each FPC is shown for weekends (A) and weekdays (B) with red (pluses) indicating the positive direction and blue (minuses) indicating the negative direction. Variance explained by each FPC: Weekends: 33.99%, 21.80%, 16.67%. Weekdays: 36.93%, 20.16%, 18.58%.

The FPCA results for intensity in the group of cats with DJD were supported by the results of for the confirmatory group (Figure 8). It is worth noting that the confirmatory group included 33 cats, a smaller number than used for the exploratory FPCA, and yet results are quite similar (Figure 10). Correlations between the scores for weekends and weekdays were 0.60 for FPC1, 0.31 for FPC2, and 0.41 for FPC3. For both weekends and weekdays, the variance about the mean for FPC1 shows a sign change at the beginning and end of each peak. Cats that are positively loaded on FPC1, have lower than average activity during early mornings in weekdays, higher than average activity between 10am and 3pm, and lower again after 3pm.

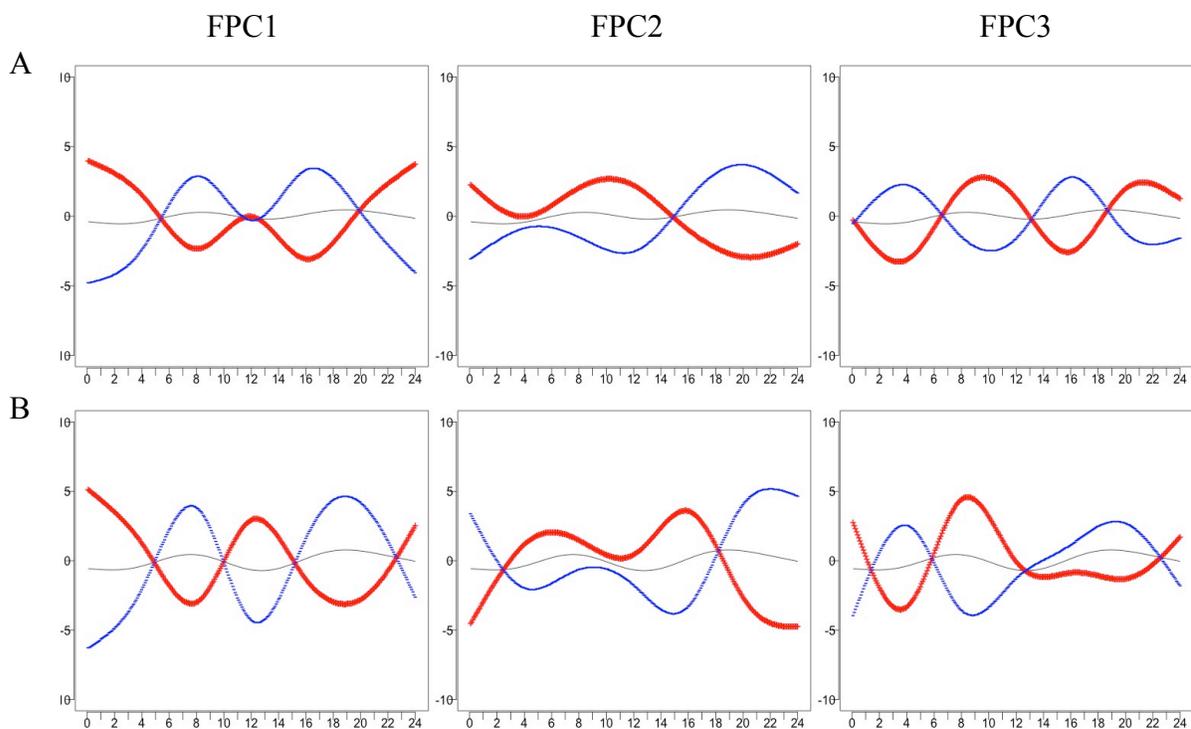


Figure 8. FPCA for Intensity in the confirmatory group. Variance about the mean corresponding to each FPC is shown for weekends (A) and weekdays (B) with red (pluses) indicating the positive direction and blue (minuses) indicating the negative direction. Variance explained by each FPC: Weekends: 31.48%, 25.16%, 20.68%. Weekdays: 34.80%, 28.56%, 17.56%.

Results of functional regression analysis evaluating the effects of age and BCS on activity and intensity profiles for weekends and weekdays in the Normal group are shown in Figures 9 (activity) and 10 (intensity). Specifically, each panel depicts the estimated effect of Age, BCS and their interaction in the solid line, as well as their 95% point-wise confidence intervals (CIs) constructed using bootstrap methods (N=2000). Results are significant when the bounds of both CIs are above or below zero; no significant effects were found for intensity profiles for weekends or weekdays. For activity profiles, age, was significantly associated, though the pattern differed over time. Older cats were more likely to be less active in the mornings on weekends, and the afternoons on weekdays.

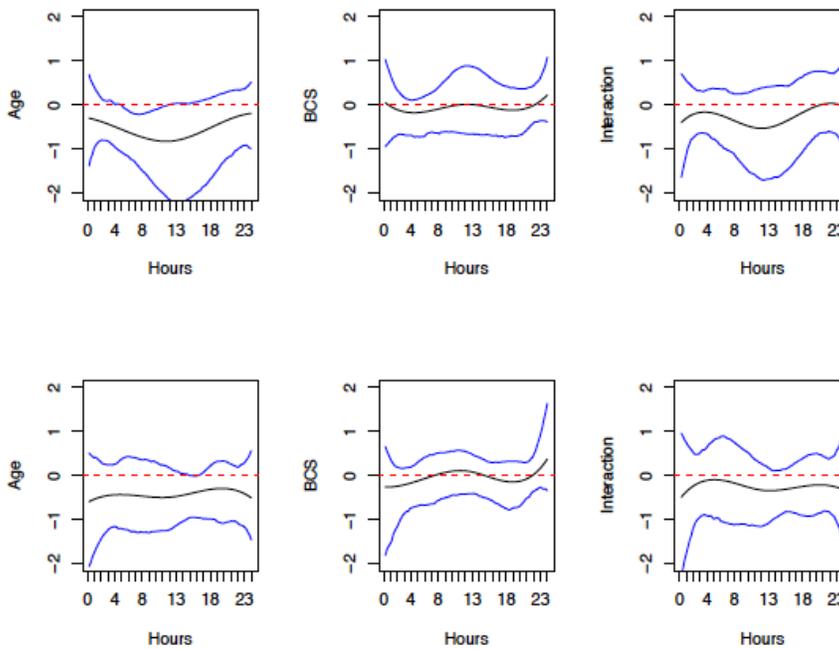


Figure 9. Depicted are the smooth effects of Age (left panels), BCS (middle panels) and AGE*BCS (right panels) on the activity of Normal cats, when model (1) is assumed. Results are shown for weekends in the top row and weekdays in the bottom row, with functional coefficients in black, 95% confidence intervals in blue, and zero demarcated in red.

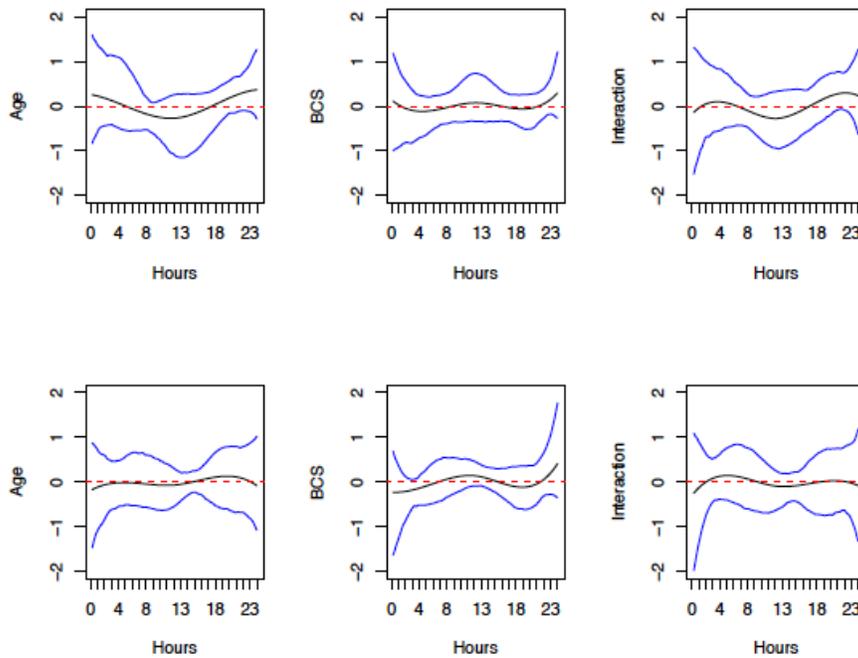


Figure 10. Depicted are the smooth effects of Age (left panels), BCS (middle panels) and AGE*BCS (right panels) on the intensity of Normal cats, when model (1) is assumed. Results are shown for weekends in the top row and weekdays in the bottom row, with functional coefficients in black, 95% confidence intervals in blue, and zero demarcated in red.

Results of functional regression analysis evaluating the effects of age, BCS, TDJD score, and TPain score on activity and intensity for weekends and weekdays in the DJD cats are shown in Figures 11 (activity) and 12 (intensity). Specifically, each panel depicts the estimated effect of Age, BCS and their interaction, TDJD score, and TPain score in solid line, as well as their 95% point-wise CIs constructed using bootstrap methods. Again, results are significant when the bounds of both CIs are above or below zero; no significant effects were found for intensity for weekends or weekdays. For activity profiles, age and pain score were significantly associated, though the pattern differed over time. During the morning and afternoon peaks, older cats were more likely to be less active on both weekends and

weekdays. However, cats with higher TPain score were more likely to be more active during the daytime hours on both weekends and weekdays.

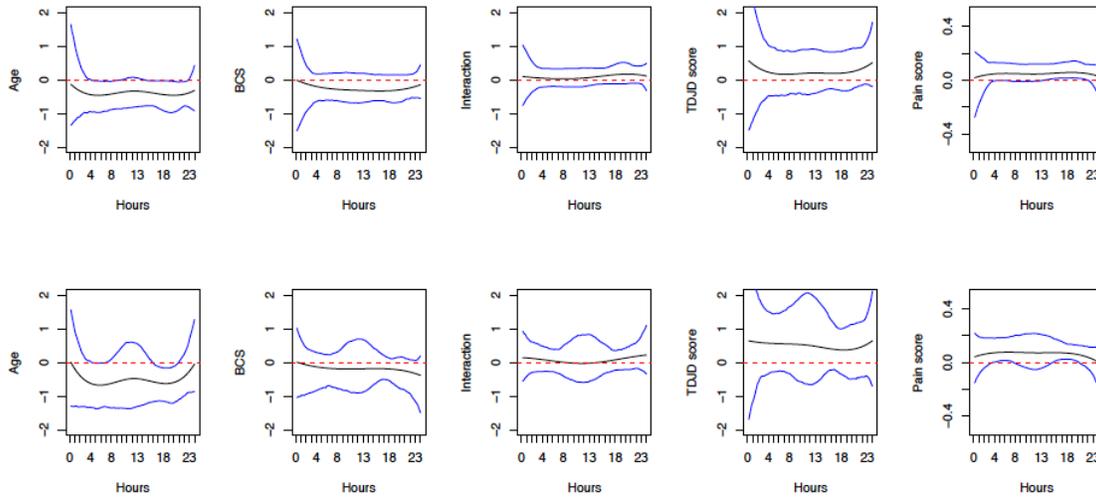


Figure 11. Depicted are the smooth effects of Age, BCS, AGE*BCS, TDJD score, and TPain score on the activity of DJD cats, when model (2) is assumed. Results are shown for weekends in the top row and weekdays in the bottom row, with functional coefficients in black, 95% confidence intervals in blue, and zero demarcated in red.

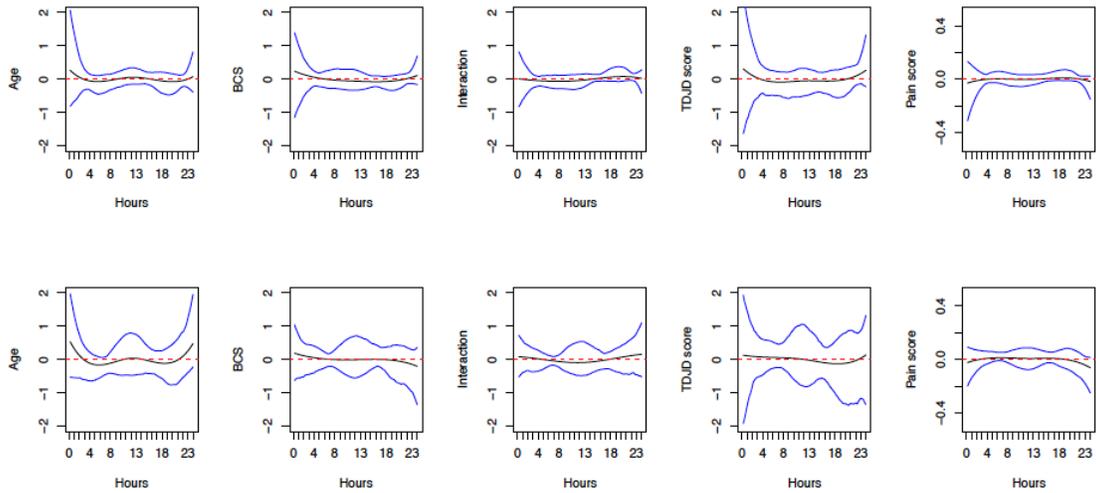


Figure 12. Depicted are the smooth effects of Age, BCS, AGE*BCS, TDJD score, and TPain score on the intensity of DJD cats, when model (2) is assumed. Results are shown for weekends in the top row and weekdays in the bottom row, with functional coefficients in black, 95% confidence intervals in blue, and zero demarcated in red.

Comparison of activity profiles between Normal cats and those with DJD

Likelihood ratio tests were used to assess the null hypothesis that activity profiles during weekends and weekdays were the same between the Normal cats and those in the DJD group. Separate tests were run with no covariates and controlling for covariates; p-values for results are summarized in Table 3 and show a difference in intensity profile between Normal cats and those with DJD during the weekdays, and a difference in activity profile between Normal cats and those with DJD on the weekends when controlling for covariates. The results indicate different mean intensity during weekdays for the two groups; for the other group comparisons, the difference in the way the response varies seem to be more complex.

Table 3. Results of likelihood ratio tests for weekend and weekday activity profiles and intensity profiles when comparing Normal and DJD cats. Models were tested both with and without covariates. Values in bold are significant after adjustment for multiple comparisons using Bonferroni correction.

	No covariates	Age, BCS, Age*BCS interaction	Age, BCS, Age*BCS interaction, and TDJD score	Age, BCS, Age*BCS interaction, TPain score
Weekend <i>activity profile</i>	0.6648	<0.0001	<0.0001	0.0320
Weekend <i>intensity profile</i>	0.9098	0.9900	0.9900	0.9999
Weekday <i>activity profile</i>	0.9999	0.0292	0.0880	0.6736
Weekday <i>intensity profile</i>	<0.0001	<0.0001	<0.0001	<0.0001

Finally, we investigated whether the way the activity varies (the population distributions) is the same in Normal cats and DJD cats; for this we used the functional Anderson-Darling testing procedure of Pomann et al.⁵⁴ We found significant evidence against this null hypothesis for both weekends (p=0.013) and weekdays (p=0.010). The same null

hypothesis was investigated for intensity and the results were also significant for both weekends ($p < 0.01$) and weekdays ($p < 0.01$). Based on these findings we conclude that the Normal cats and DJD cats show different levels of activity both during the weekends and weekdays, and also show different intensity of activity. This test indicates that the distributions are different, without providing information on how they are different. Visual inspection of the mean functions (Figure 13) shows that they cross each other at various times of the day, with the Normal cats having more variable activity and intensity, while the activity and intensity profile of the cats with DJD appears muted across the day.

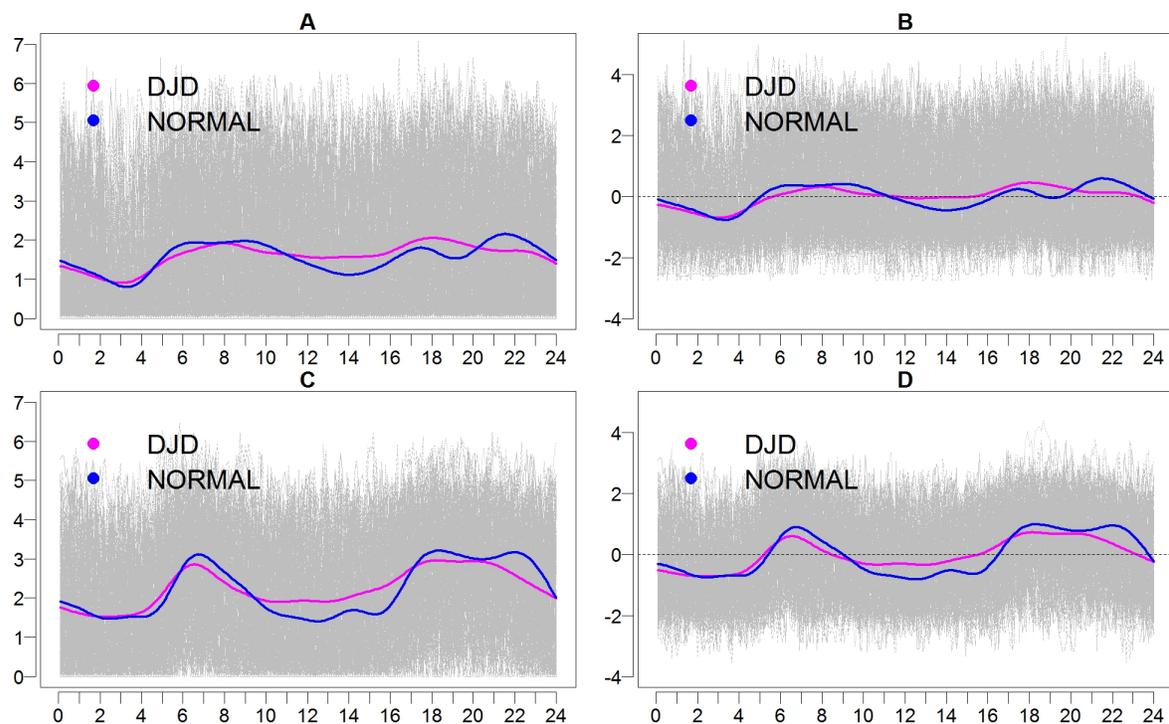


Figure 13. Activity (left) and intensity (right) profiles for cats in the Normal group (blue) and those with DJD (pink). Log transformed activity for all cats is shown in gray with time (in hours) along the horizontal axis. The group mean for activity and intensity are shown for weekends (A&B, respectively) and weekdays (C&D, respectively).

Discussion

In this study, functional data analysis methods were used to examine the pattern of activity and intensity in cats with and without DJD. This represents a novel method of analysis, one that allows for further understanding of spontaneous activity profiles in cats, and how age, pain, and radiographic DJD affect activity profiles in this spontaneous model of DJD across the day. This understanding is critical to our ability to use activity as an effective objective outcome measure, both for monitoring an individual cat longitudinally, but also in response to a therapeutic intervention. As cats represent a model of naturally-occurring DJD, further understanding of activity can expand our ability to investigate treatment options that may be of benefit to humans as well as cats. Through FDA, we now understand how DJD-associated pain alters the spontaneous activity profile, and points the way forward to how to assess the effects of analgesic treatments in a sophisticated and elegant manner, rather than looking at a coarse summary variable such as mean activity per minute per unit time period.

Using a group of normal cats, without DJD and associated pain, as well as cats with varying degrees of DJD, this study identified significant differences between the activity pattern of cats during the weekdays and weekends. These differences are important as it highlights a point raised by Piccione et al regarding the influence of human activity on the activity pattern of cats.^{39,40} Cats are generally defined as diurnal or crepuscular, with peaks of activity at dawn and dusk,^{57,58} a pattern that matches the bimodal pattern seen in this study. However, cats may readily adapt to different housing conditions. In an intriguing study done in 2013, Piccione showed that cats that are kept outdoors overnight have a strikingly different pattern of activity, and an increase in overall activity, compared to cats that are kept indoors

overnight.⁴⁰ The authors of that study proposed that human activity was the major influencing factor on the pattern of cat activity for cats housed exclusively indoors. The current study supports and extends these findings by showing that activity on weekends, when owners typically have an altered schedule, is different from weekdays. Here, the weekend activity across all groups of cats had more muted peaks with the morning peak shifted to the right (later) suggesting that the morning activity began later on weekends. This could be explained by a strong influence of owner activity on cat activity. As owners are more likely to wake up and begin daily routines at more variable times on weekends, each cat's peak of activity would be less uniformly distributed, contributing to the flattening of the peak. However, on weekdays, where owners are more likely to get up earlier and leave the house at more uniform times given typical working hours for many, activity related to caretaking (feeding, medication, play, etc.) is more likely to be concentrated in the morning, and may be less uniform in the evening, as owners return home at different times and may interact with their cat in a variety of ways over the hours between returning home and retiring to bed. This is supported by the narrower peak seen in the morning and the broader evening peak seen on weekdays. For the cats in these studies, demographic information collected from owners quantified how many hours owners were away from home, but not the details of when those hours occurred, making it difficult to explore whether there was a difference in the distribution of activity for cats whose owners were out of the home during working hours versus those that were not. Work is currently underway to explore this relationship by having a cohort of cats wear activity monitors while owners detail their times in and out of the home, as well as their interactions with their cats around food, play, and social interactions. While this should be further explored in studies that account for owner schedules, it suggests that

when using activity data from cats, the number of weekend and weekdays should be standardized across data sets. This has also been suggested by studies in humans⁵⁹ and dogs.²³

The differential effect of weekday and weekend on activity is particularly important to take into account in evaluating treatment response. One study performed in laboratory cats²⁹ selected nighttime activity on weekends in order to decrease the effect of the human caretakers on spontaneous activity, thus attempting to focus on the activity modulating effects of the analgesic drugs being administered. This approach is interesting, and certainly decreases variability as cat – caretaker interaction can differ, but may underestimate the potential effect of the analgesic to increase activity in response to human interaction. If cat activity is heavily influenced by human activity, then hypothetically, analgesic treatment could lead to increased interest in interactions and thus increased activity (Figure 14).

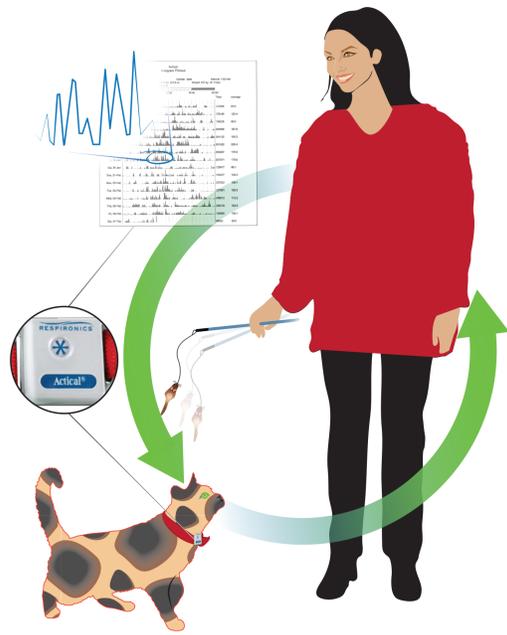


Figure 14. Relationship between human activity, increased interactions, and increased activity and activity counts for cats with DJD receiving analgesic therapy.

In addition to the differences in activity profiles over weekends and weekdays, activity profiles and intensity profiles are different between Normal cats and those with DJD. However, it is not as simple as finding that the activity and intensity are consistently higher for the Normal cats as opposed to those with DJD. Indeed, a direct comparison of mean activity counts per minute in Normal cats versus cats with DJD showed no difference in activity. However, when using FDA to evaluate activity patterns, we found that cats with DJD appear to have higher activity and intensity of activity at some times during the day, while the height of their peaks appears flattened compared to the Normal cats at other times. Overall, the variation in activity over the day appears to be muted, with lower peaks, and less deep troughs, in cats with DJD compared to Normal cats. Cats typically experience bouts of activity in spurts rather than sustained trotting or running as might be seen in dogs. It is possible that the height of the peaks for the Normal cats represents relatively more of these

bursts of activity, so that what may be important is the height and number of the peaks. In humans, age has been associated with an increase in low-intensity activity at the expense of high-intensity activity, with high-intensity physical training in older persons resulting in a compensatory decrease in low-intensity activity.⁶⁰ As age is associated with chronic pain, this compensatory relationship may be pain related.

Cats experiencing joint-related pain may show a similar decrease in the number of spurts of activity, while maintaining a more consistent level of low-intensity activity. Pain-induced restlessness could contribute to this low-intensity activity, and this deserves further investigation. While work has been done in dogs to establish cut-points for distinguishing intensity of activity,⁶¹ such work has not been done in cats. Age has previously been shown to be associated with decreased activity in both cats and dogs,²³ and cut-points in accelerometer counts for defining intensity of activity may need to reflect changes in baseline activity that occur with age. In the present study, functional regression showed that in the DJD groups, older cats were more likely to be less active across the majority of the day on both weekends and weekdays, but specifically in the morning and afternoon. Also in the DJD groups, cats with higher pain scores on orthopedic exam were more likely to show increased activity during the morning and afternoon. The reason for this is unknown, but may be related to the incongruency between pain on veterinary orthopedic exam and decreased mobility/activity in the home. Results from the FPCA suggest that morning and evening peaks (the mean behavioral pattern) account for the majority of variability. The first dominant mode of variation for both Normal and DJD cats represents the morning and evening peaks as having a different pattern of variation from the rest of the day (during

weekdays). This variation pattern explains almost half of the total variance (~40% for each) and was confirmed in the analysis of the fourth group of cats. While the FPCs for weekends and weekdays are not controlled for cats (i.e. cats may be loaded positively on FPC1 for weekends and negatively loaded for FPC1 on weekdays), their scores are positively correlated indicating that if a cat is positively loaded on a component for the weekend, they are likely to be positively loaded on that component for weekdays.

Additional areas of interest in understanding activity patterns in cats include 1) defining “normal” activity for a cat of a given age or health status would be valuable for determining an individual’s status relative to a population norm for their age, and 2) being able to use baseline activity to stratify cats for randomization in clinical trials. Prior to log transformation of the data, ranges, means, and medians of average per-minute activity across the period were generated, and showed similarity between the means and medians for all groups of cats. While FDA showed that the mean activity profile over the day was different at times, it would not be possible to classify a cat as normal or abnormal based only on their activity counts. Therefore, the first goal does not appear possible; cats show variability in activity independent of DJD and pain, similar to variability seen in people, though additional studies with larger numbers of cats should be performed. However, using the median average per-minute activity, the second goal is potentially achievable. In general, studies of therapeutic interventions for DJD have randomized or stratified based on an owner rating³⁰ or radiographic DJD,²⁹ and then used activity as the objective outcome measure. Median per-minute activity could be used as a variable for randomization to a clinical study group, or

even as an entry criterion for early clinical studies, assuming that lower median activity indicates pain-related decrease in activity.

Limitations and future work

This study was designed to evaluate differences in activity patterns between a group of Normal cats and a group of cats with DJD using functional data analysis. However, several limitations exist which warrant discussion. First, the group of Normal cats was smaller in number and significantly younger than the group of cats with DJD. In contrast, the cats with DJD were necessarily more impaired, as these were cats selected for inclusion in clinical trials, with a relatively high bar set for entry. While these groups of cats were not matched for age, and age is clearly important in physical activity level, it is difficult to find older cats without radiographic DJD (and associated pain) as prevalence of radiographic DJD in cats has been estimated at 60-92% of cats, with increased prevalence associated with age.^{62,63} The current study required cats that were classified as Normal to have minimal to no radiographic evidence of DJD. As radiographic disease does not correlate perfectly with the presence of pain,⁶⁴ this study further required that cats defined as Normal have minimal to no pain on orthopedic exam. This qualification was required as it is not yet known what degree of pain on orthopedic exam or radiographic DJD corresponds to clinically relevant pain or mobility impairment. Indeed, a study by Guillot et al.²⁹ included a group of cats classified as having abnormal orthopedic exam findings but no radiographic evidence of OA, and these cats were not impaired on peak vertical force, a measure generally considered more sensitive than simple observation, suggesting that pain on orthopedic exam may not translate to clinical signs of impairment. Given the discrepancies between the groups of cats, future work

to understand activity patterns in cats should use a randomly selected group of cats of varying ages and phenotype them following the collection of activity data. This would allow better understanding of whether there is a breakpoint for pain on exam or radiographic DJD that predicts lower activity.

Still, given the dichotomy of the two populations used in this study, it is even more striking that the activity patterns were not more distinct between the Normal and DJD cats. In cats, as in dogs and people, there exists a wide variation of activity levels. For clinical trials, it may be possible to randomize cats based on baseline activity, but the inter-cat variability and generally small number of cats enrolled in clinical trials suggest that cats will continue to need to be evaluated as their own controls for intervention trials. In this study, the Normal cats had a more restricted range of per-minute activity than any of the other groups of cats, but this could be due to the smaller number of cats in this group, and expansion of this group could show a wider range of per-minute activity, though frequently smaller numbers are associated with greater variability. While all activity monitors were worn in the same manner, mounted on a neck collar, the same set of activity monitors was not necessarily used in each study. While laboratory based validity calibration has been performed for activity monitors in cats,²² reliability calibration is not routinely performed outside of that provided by the manufacturer at intermittent times. Inter-accelerometer variability has been shown to be higher than intra-accelerometer variability,⁶⁵ and unpublished data from our collaborator suggests that while the activity monitors are internally consistent, some may register activity counts at lower acceleration, resulting in higher activity counts. This may be accounted for in our use of intensity, which compensates not only for inter-cat variability, but also for

uncalibrated accelerometers or varying output from accelerometers. Using intensity, this study did not show significant effects for covariates within a group, but did find significant differences between the Normal cats and those with DJD during the weekdays.

Conclusions

This type of functional data analysis is novel for activity data in companion animals. The functional principal components analysis employed here was exploratory in nature, but confirmed by the fourth group of cats. The similarities between the FMPI and Low-dose studies suggest that the differences found between the Normal cats and those with DJD are real differences, but this should be explored more in future work. In addition, future work should evaluate the change in activity pattern in response to an analgesic therapy. While several studies have shown that analgesics can increase activity in cats with DJD,^{25,29} these studies have all used average per-minute activity over a treatment period. Functional data analysis can help us understand the pattern of these improvements. Based on the results of this study, we suggest that changes in activity in response to an analgesic might be most apparent during the morning and evening peak on the weekdays, when the activity of cats with DJD appears lower than that of cats without DJD, and has the opportunity to increase in response to interactions with owners. However, alternatives to this suggestion are possible, and this will be an area of future research.

Further work that incorporates owner schedules will increase the granularity of analyses, and can shed light onto the effect of owner presence on activity peaks and on increased activity in response to an analgesic. Indeed, the previously discussed placebo-by-

proxy effect could be further explored using functional data analysis, as activity in cats should increase more when their owners are home if this effect is beneficial. Of great benefit to the understanding of activity in cats would be a longitudinal study of cats over their lifetime, from youth through to advanced age, correlated with changes in pain, radiographic DJD, weight, and health status. This type of study should also include additional subjective assessments of cats, including observations of temperament and behavior. It is possible that the interaction between pain, radiographic DJD, and activity is complex, as is seen in humans, and that behavioral traits interact both with baseline activity as well as response to DJD and associated pain. Future work to better understand temperament traits in cats is ongoing, and incorporation of these findings into studies of activity and treatment response will deepen our interpretation of results.

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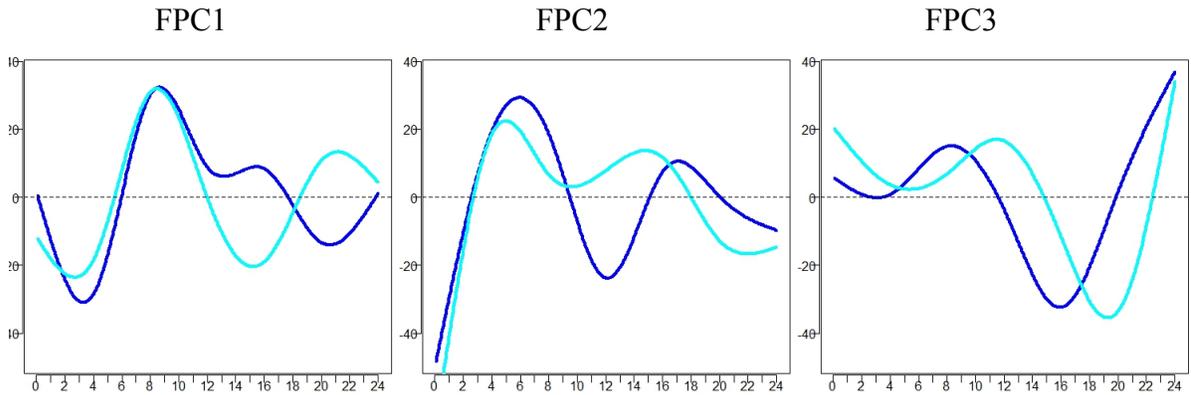
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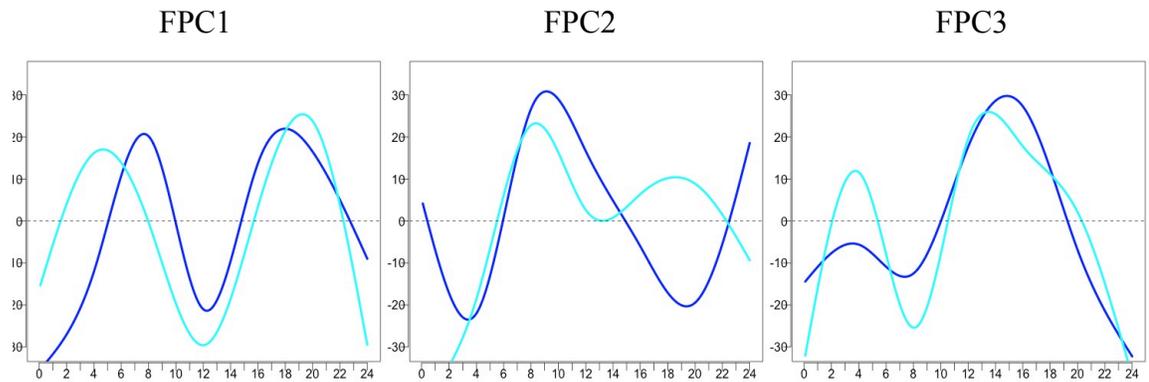
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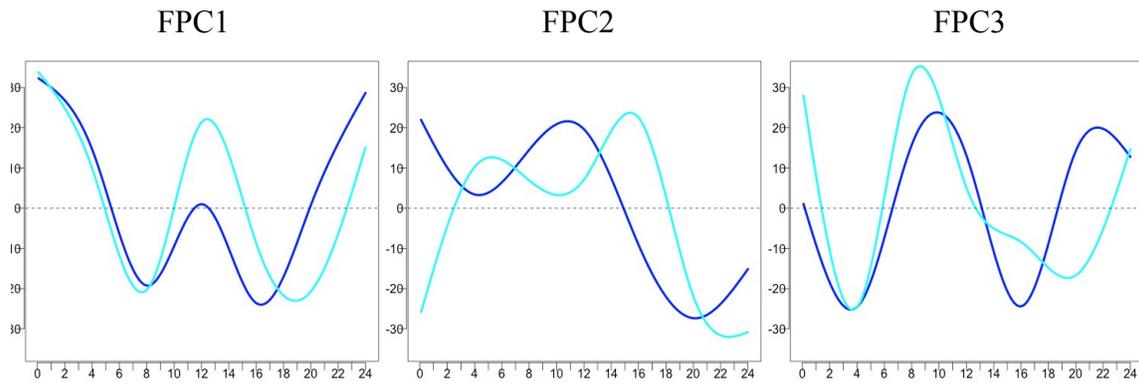
Supplemental figures:



Supplementary Figure 1. Results of FPCA for Intensity for the Normal cats. (A) The top three eigenfunctions of intensity profiles for activity during the weekend (black) and weekday (blue). The first component describes $\sim 40\%$ of the total variance for both weekends and weekdays and picks up the two peaks seen in the activity profiles.



Supplementary Figure 2. Results of FPCA for Intensity for the DJD cats. The top three eigenfunctions of intensity profiles for activity during the weekend (dark blue) and weekday (light blue). The first component describes $\sim 34\%$ of the total variance for the weekends and $\sim 37\%$ for the weekdays and picks up the two peaks seen in the activity profiles.



Supplementary Figure 3. Results of FPCA for intensity in the confirmatory group. The top three eigenfunctions of intensity profiles for activity during the weekend (dark blue) and weekday (light blue). The first component describes ~35% of the total variance for both weekends and weekdays and picks up the two peaks seen in the activity profiles.

**Chapter 4b. A Comparison of Serum and Plasma Values using a Multiplexed Cytokine
assay in Cats**

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Introduction

Cytokines and chemokines as biomarkers are of great interest, and in veterinary medicine, development of biomarkers for disease is an area of active research and growing literature base. Many studies in human and veterinary medicine have begun to explore and identify cytokines involved in a variety of disease processes, however methodological issues and a lack of standardization have contributed to inconclusive or contradictory findings. The advent of multiplex technologies that can measure a panel of cytokines and chemokines in small volumes of biological samples has increased the ability to screen for potential biomarkers and targets for further research, however concerns have been raised about the ability to compare results from studies using differing assays and media.¹⁻⁴

Cytokines are proteins that are produced by a variety of cells, particularly the T-lymphocytes and macrophages, and are integrally involved in inflammation and cell signaling. The vast majority of cytokines are produced and consumed locally, acting in autocrine, paracrine, and juxtacrine manners. This makes detection of cytokines in systemic circulation challenging, and findings from the serum or plasma might not be reflective of local tissue activity. Despite this, obtaining whole blood is more convenient than most other sampling modalities, particularly in clinical cases, and offers a potential for screening large populations. In fact, concentrations of several cytokines have been found to be altered in the serum and plasma of patients during disease, including IL-6 in humans with rheumatoid arthritis³ and dogs with immune-mediated polyarthritis,⁵ and stromal cell-derived factor-1 in horses with osteochondral injury.⁶ These reports pertain to serum or plasma samples, but the two sample types are not necessarily interchangeable.

Several recent studies have investigated the relative difference in multiplex measurements of cytokines between serum and plasma from humans, and have demonstrated a generally high correlation, but have identified issues including matrix impedance and non-specific background binding that is not completely eradicated by the use of manufacturer-provided buffer solutions.⁷ In addition, the anti-coagulant used to obtain plasma can affect the concentrations of certain cytokines.⁸ Processing and sample handling post-blood draw can further complicate findings as time to separation of serum or plasma, storage, and number of freeze-thaw cycles have all been shown to affect cytokine concentrations.^{2,4,8,9} Even when these methodological issues are controlled by common treatment of samples, the optimal medium could be cytokine specific.² When using commercially available multiplex kits with a panel of cytokines, selection of serum or plasma might depend on the relative benefits of one over the other, including number of detectable samples, and the ability to observe a difference in a group with a known disease as compared to a control group. While it has been noted that serum and plasma are not interchangeable for multiplex assays,¹⁰ to our knowledge the comparison of multiplex-measured cytokine concentrations in serum versus plasma have not been assessed in cats. Therefore, the objective of this study was to compare the concentrations of 19 cytokines in matched serum and plasma samples from cats using a commercially available multiplex magnetic bead assay and analysis software in order to guide the selection of medium (serum or plasma) for future work.

Materials and Methods

Subjects: Matched serum and plasma samples were used from 38 cats that had been screened for one of three clinical studies at the North Carolina State University College of

Veterinary Medicine (NCSU-CVM). All cats were examined under approval from the Institutional Animal Care and Use Committee at NCSU.

Serum and plasma were collected from each cat during a single visit to the NCSU-CVM. For serum samples, whole blood was collected into a 3mL anti-coagulant free glass tube (red top) and allowed to clot at room temperature for at least 30 but no more than 60 minutes. Clotted samples were centrifuged at 1163 x g for 10 minutes, and serum was removed, aliquoted, and stored in cryovials at -80°C until use. For plasma samples, whole blood was collected into a 2.7 mL glass tube (lavender top) containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The volume of blood added was according to manufacturer recommendation to achieve a concentration of EDTA of approximately 1.8 mg/mL of blood. Within 30-60 minutes of collection, samples were centrifuged at 1163 x g for 10 minutes and plasma was similarly processed and stored. All samples had a maximum of one freeze-thaw cycle prior to use; while multiple freeze-thaw cycles can affect cytokine concentrations and should be avoided, one freeze-thaw has been shown to have minimal effects on measured cytokine concentrations in previous studies.^{8,9}

Feline-specific cytokine kits (19-plex)^a were purchased and used according to the manufacturer's recommendations. Quality control samples were run on each plate, in duplicate, and sets of serum and plasma (in duplicate) were run on the same plate (i.e. no pairs of serum or plasma for an individual cat were run on different plates). Plates were analyzed using a dedicated plate reader and software^{b,c}.

Results were analyzed using dedicated software;^{c,d} bead count, standard curves, quality control samples, and coefficients of variation (CVs) for each analyte were assessed. Quality controls were compared with the range provided by the kit. As per manufacturer's recommendation and industry-accepted standards for multiplex assays, CVs less than or equal to 20% were considered acceptable. In cases where CVs were >20%, no value for that sample was recorded, and the number of samples for that analyte were therefore reduced. Reported concentrations for each analyte and each sample (cat) represent the mean of the duplicate samples.

For each sample type (serum and plasma), detection was reported as the number of results that were in range, those below or above the limit of quantification (LOQ), and the number of analytes that had >25% and >50% of the results below the lower LOQ (LLOQ). Correlations between the serum and plasma results for each analyte were generated using Pearson correlation coefficients for any analyte with ≥ 9 serum/plasma pairs in range (25% of total pairs). To evaluate overall differences, a repeated measures model (ANOVA) was used with fixed effects for analyte, sample type (serum or plasma) and plate (1 or 2), and a random subject effect. To evaluate the effect of sample age, a repeated-measures model was used with random effects for subject and plate, fixed effects of the analyte and age of sample (in days), and a response variable of the ratio of serum: plasma. To evaluate the consistency of results across cats, the ratio of serum: plasma results for each cat and each analyte was tabulated. Where appropriate, a p-value of 0.05 was considered significant, and statistical analysis performed using a standard statistical software program.^e

Results

Subjects

Matched serum and plasma were available for 38 cats that had been screened at the NCSU-CVM. Cats were screened for participation in studies of normal cats as well as treatment trials for degenerative joint disease. All samples were obtained during the period from 2007-2014, and were taken prior to any treatments being given. Cats were between 1 and 19 years of age (mean 11.5 years) and 2.08-8.46 kg (mean 4.9 kg).

Serum and plasma comparisons

All quality control results were within range with acceptable CVs. Results were removed for low bead counts for 72 individual results (5.0%) across all the samples and cytokines measured, however these were generally clustered within a sample, so these 72 wells represented results from five cats. Overall, CVs and bead counts were generally within acceptable range for all of the analytes measured, although 6.8% of the plasma samples and 6.1% of the serum samples were removed from the analysis due to either low bead counts or high CVs. The majority of CVs were below 15%.

The number of results that were in range (above the LLOQ) for each analyte and each matrix are shown in Tables 1 and 2. No results were above the upper LOQ. Correlations between serum and plasma were calculated for 11 analytes with 9 or more pairs of results, and are shown in Table 3. Correlation coefficients were generally positive; six of 11 analytes (55%) had correlation coefficients >0.9 , however correlation coefficients were below 0.9 for five of the 11 analytes (45%). The ratio of serum: plasma results for each analyte and each

cat are shown in Figure 1. The ratios show the tendency for serum to be higher than plasma, but this varied by analyte and by cat within an analyte.

Table 1. Number of results above (in range) and below the lower limit of quantification (LLOQ) for each of the 19 cytokines measured.

	Plasma	Serum	Count		Plasma	Serum	Count
FAS	In range	In range	4	IL-6	In range	In range	9
	↓LOQ	In range	1		↓LOQ	In range	3
	In range	↓LOQ	0		In range	↓LOQ	0
	↓LOQ	↓LOQ	31		↓LOQ	↓LOQ	22
FLT-3L	In range	In range	35	IL-8	In range	In range	7
	↓LOQ	In range	0		↓LOQ	In range	18
	In range	↓LOQ	0		In range	↓LOQ	0
	↓LOQ	↓LOQ	0		↓LOQ	↓LOQ	11
GM-CSF	In range	In range	2	CXCL1	In range	In range	3
	↓LOQ	In range	0		↓LOQ	In range	11
	In range	↓LOQ	0		In range	↓LOQ	0
	↓LOQ	↓LOQ	34		↓LOQ	↓LOQ	20
IFN-γ	In range	In range	9	MCP-1	In range	In range	22
	↓LOQ	In range	4		↓LOQ	In range	6
	In range	↓LOQ	0		In range	↓LOQ	2
	↓LOQ	↓LOQ	20		↓LOQ	↓LOQ	5
IL-12 p40	In range	In range	35	PDGF-BB	In range	In range	0
	↓LOQ	In range	0		↓LOQ	In range	0
	In range	↓LOQ	0		In range	↓LOQ	0
	↓LOQ	↓LOQ	0		↓LOQ	↓LOQ	35

Table 1 (continued).

IL-13	In range	In range	17	RANTES	In range	In range	30
	↓LOQ	In range	3		↓LOQ	In range	5
	In range	↓LOQ	1		In range	↓LOQ	0
	↓LOQ	↓LOQ	15		↓LOQ	↓LOQ	0
IL-18	In range	In range	28	SCF	In range	In range	26
	↓LOQ	In range	1		↓LOQ	In range	5
	In range	↓LOQ	1		In range	↓LOQ	0
	↓LOQ	↓LOQ	4		↓LOQ	↓LOQ	3
IL-1β	In range	In range	5	SDF-1	In range	In range	21
	↓LOQ	In range	2		↓LOQ	In range	4
	In range	↓LOQ	0		In range	↓LOQ	2
	↓LOQ	↓LOQ	27		↓LOQ	↓LOQ	10
IL-2	In range	In range	6	TNF-α	In range	In range	6
	↓LOQ	In range	0		↓LOQ	In range	3
	In range	↓LOQ	0		In range	↓LOQ	0
	↓LOQ	↓LOQ	29		↓LOQ	↓LOQ	27
IL-4	In range	In range	17				
	↓LOQ	In range	4				
	In range	↓LOQ	1				
	↓LOQ	↓LOQ	13				

Table 2. Analytes with less than 50% and less than 25% of samples within range (above the lower LOQ) for serum and plasma.

<25% Serum (<9)	<25% Plasma	<50% Serum (<19)	<50% Plasma
FAS	FAS	FAS	FAS
GM-CSF	GM-CSF	GM-CSF	GM-CSF
IL-1β	IL-1β	IFN-γ	IFN-γ
IL-2	IL-2	IL-1β	IL-13
PDGF-BB	IL-8	IL-2	IL-1β
	CXCL-1	IL-6	IL-2
	PDGF-BB	CXCL-1	IL-4
	TNF-α	PDGF-BB	IL-6
		TNF-α	IL-8
			CXCL-1
			PDGF-BB
			TNF-α

Table 3. Correlations between serum and plasma results for each analyte with 9 or more pairs of results.

Analyte	N	Pearson's Correlation	P-value
FLT 3L	35	0.945	<0.001
IFN- γ	9	0.893	0.001
IL-12p40	35	0.724	<0.001
IL-13	17	0.990	<0.001
IL-18	28	0.561	0.002
IL-4	17	0.931	<0.001
IL-6	9	0.948	<0.001
MCP-1	22	0.416	0.054
RANTES	30	0.973	<0.001
SCF	26	0.954	<0.001
SDF-1	21	-0.010	0.966

	FAS	FLT3	GM-CSF	IFN- γ	IL-1 β	IL-2	PDGF-BB	IL-12	IL-13	IL-4	IL-6	IL-8	CXCL-1	SDF-1	RANTES	SCF	MCP-1	TNF- α	IL-18
Aramis		1.00						0.93							1.08				0.82
Biscuit		0.92		0.94				0.89	1.11					30.45	1.32	1.56	0.91		1.19
Bruce	0.82	0.88	0.70			0.83		0.85	0.87	0.70					0.84	2.59	0.79	0.74	0.84
Bruiser		1.40		1.84	1.47	1.24		1.47	1.18	1.43	1.53	1.41	14.73	1.27	1.52	1.47	1.32	1.58	1.44
Bunker		1.25		1.84				1.24		1.30					0.73	2.04	1.33	0.02	0.99
Caol Ila		1.68			2.29			1.96	2.04										0.77
Chester		1.05			1.23			1.08		1.50	1.06				1.45			1.71	0.94
Chloe		1.16		1.19		1.00		1.04	1.02	1.13			1.19	92.28	1.64	1.13	0.01		1.05
Cookie		1.50						1.31							1.39	1.70			0.83
Cosmo		1.20		1.52	1.37			1.26	1.12	1.03	1.24	1.65		1.20	2.22	1.27	1.19	1.48	
Diablo		1.43						1.63	1.32	1.83		1.93		1.28	1.68		1.19		1.06
Ellie	1.03	1.06				1.04		0.97	1.14	1.14	1.00	1.02		1.05	1.24	1.06	1.00	1.11	0.92
Elvis		0.58						0.51			1.01				1.01		0.82		0.95
Jasper		1.15						1.19							1.38				
KC		1.61				0.95		1.53						1.63		1.64			
Lemur	2.99													3.57					
Lily		3.67						3.27								4.38			
Lucky																			
Magic		0.92						0.99	0.97	0.86				0.77	0.65	0.84	0.79		1.14
Malachi								1.37						0.11	0.05				
Mister		2.34		1.88				2.56	1.25	2.22				0.49	2.43	2.59	2.35		0.02
Monterey Jack		1.62						1.55							2.16	169.48			1.91
Moose		1.48						1.57						1.61	2.24	2.07			
Muffin		1.95						1.74	0.80	1.83				5.42	1.90	0.78			0.85
Nother		1.25		1.33				0.01	1.06	1.14		3.84		1.31	2.94	1.15	1.25	2.19	0.92
Otis		8.83						6.67											2.16
Pee Dee		1.44						1.29		1.50					1.45		1.40		1.65
Rat		1.64						1.37							3.25	1.78	0.75		1.13
Sade		1.41						1.30	1.14	1.32	1.19				3.35	1.45	1.05		0.91
Scout		2.68						2.64	2.85						6.51	3.46	0.01		1.66
Sophie	1.89	1.60	1.65	2.58		1.42		1.51	1.39	0.60	2.82			1.82	280.67	1.79	2.17		0.02
Stanley		1.44						1.44			1.55			1.47	2.25	1.51	1.13		
Tabi		1.37						1.20						1.23		1.31			
Tasha		1.77						1.50				2.40			2.44	1.48			1.03
Teddy		1.44						1.71	1.33					1.62	1.44	1.34	1.13		0.80
Tessa		1.02		1.22	1.12			0.91	1.21	1.11	1.08	1.23		1.00	1.08	1.25	1.07	1.19	1.02
Thunder		1.60						1.48									0.81		0.96
Trinity		1.23						1.42						0.22	450.25	1.49	1.21		1.21

Figure 1. Serum: plasma ratios for each cytokine analyte and each cat. Ratios below 1.0 are in yellow, between 1.0 and 2.0 are in green, and above 2.0 are in pink. This figure shows the variability in ratios between analytes and between individuals for any single analyte.

Results of the repeated measures model evaluating sample type showed no significant difference between plasma and serum ($p=0.620$). There was no significant sample type (plasma or serum) by analyte interaction; serum values were generally higher than plasma, regardless of the analyte being considered (Figure 1). There was also no significant effect of sample age on the ratio of serum: plasma ($p=0.510$).

Discussion

The objective of this pilot study was to compare serum and plasma cytokine concentrations in matched samples from cats using a feline specific multiplex assay. Results indicated that the correlation between serum and plasma was generally positive, and was high for over half of the analytes with a reasonable number of pairs for comparison (set at 25% of total sample pairs). However, correlations for 45% of the analytes considered were below 0.9, and ranged from -0.01 (negligible) to 0.89. Both detection and overall values were higher in the serum samples than in the plasma samples, though the effect was not significant overall or for analyte by sample interaction. In the serum samples, results were undetectable (below the lower limit of quantification) in 75% of the samples for five analytes, while this was true for eight analytes in the plasma samples. Discussion with the manufacturer of the plates revealed that median values obtained during our study were comparable to those obtained during development of the assay (for serum), and is similar to findings in humans in the absence of disease.^{2,10} Higher detection and higher concentrations of cytokines in serum over plasma have also been shown in other studies in humans,^{2,7} though occasionally the opposite has been reported.¹¹ Plasma is the fluid fraction of the blood and contains clotting factors and fibrinogen; these components are absent from serum, as they are consumed during clot formation. In the process of clot formation, however, leukocytes and platelets can be activated, leading to cytokine release, and potentially falsely elevating concentrations in serum over plasma. This has been shown for certain cytokines, including IL-1, and IL-8, but can be minimized by standardized and rapid processing of samples following clot formation.¹² Prompt processing is critical for accurate measurement in plasma samples, too,

as important effects of time to processing and storage have been demonstrated with both increased and decreased concentrations of several cytokines as samples age.¹³

The higher levels of detection in serum over plasma, and relative greater ease in obtaining serum suggest that serum would be the most suitable medium for future, large scale screening studies in cats. However, a recent study by Rosenberg-Hasson et al⁷ found that while detection was improved in serum over plasma, the sensitivity of plasma to changes in measured cytokine concentrations in multiple myeloma patients compared to non-affected patients was superior to serum. They suggest that greater background non-specific binding in serum samples masks small changes in cytokine concentrations that are better detected using plasma, and that 'higher' is not necessarily better. In contrast, Dymock et al⁶ concluded that stromal cell-derived factor 1 measured in serum was more sensitive than plasma or synovial fluid in distinguishing horses with osteochondral injury from uninjured horses. In the current study, the objective was to compare the values obtained from serum and plasma in cats, however future work should evaluate the relative sensitivity of serum and plasma to detect changes in cytokine concentrations in disease in a larger sample of cats.

In this study, evaluation of the ratio of serum to plasma for individual cats and analytes showed that the relationship between the values for serum and plasma were not consistent across analytes or across individual cats, suggesting that a simple correction factor would not be appropriate for comparing serum and plasma values for cytokines. For example, for RANTES, the one analyte where the ratios were in the same direction for all cats (serum: plasma ratio greater than 1), the ratios ranged from close to one to over six.

It is clear that sample handling and storage can affect measured concentrations of cytokines, and this has been rigorously evaluated in studies using spiked samples and various blood collection tubes and anticoagulants, and differing freeze-thaw cycles (see Zhou¹⁴ and Keustermans⁴ for reviews). Other handling concerns include timing of blood draw, as several cytokines show circadian patterns with predictable peaks.^{15,16} This is generally related to the concentration of plasma cortisol, which follows a circadian pattern in dogs¹⁷ and humans.¹⁵ While this is a potential concern, in cats it does not appear that cortisol follows the same circadian pattern, showing instead an episodic release, making effects on measured cytokine concentrations difficult to predict.^{18,19} For this study, samples were generally obtained in the morning, and the serum and plasma obtained during the same blood draw. However, effects of the timing of sampling should be evaluated in future work with cats. Other factors shown to affect measured cytokine concentrations, including feeding,²⁰ were not controlled for in this study, but are unlikely to differentially affect serum versus plasma. A further limitation of the current study is the different ages of the samples used. Storage over time, even at -80°C has been shown to decrease measured concentrations of cytokines,² but it is unknown if the level of degradation is different for serum versus plasma. In this study, the differences between serum and plasma results were not different for samples of varying ages, but it is not known what the concentrations were at the time the samples were taken.

In summary, this study found that while results from matched serum and plasma samples in cats were positively correlated, both detection (readings above the LLOQ) and measured values were higher in the serum samples. While CVs were low for the majority of the samples, high CVs did occur, which could indicate variability due to sampling error, and

supports the use of triplicate samples when running multiplex plates. The ratio of serum to plasma results was not consistent across analytes or cats, thus a correction factor could not be applied to make serum and plasma samples comparable, which supports the recommendation to select one or the other medium when running multiplex assays. While these findings are not necessarily unexpected, they do represent the first report of a comparison of serum and plasma concentrations of cytokines in samples from cats. Future work should evaluate the effects of storage on relative values for serum and plasma compared to values obtained when running fresh samples, as well as evaluate whether relative changes in cytokine concentrations are more sensitively detected in serum or plasma. This study focused on samples obtained through clinical practice, but future work to verify these findings using pooled samples of serum and plasma, and known (spiked) concentrations of cytokines.

^a FCYTMAG-20K-PMX Feline Cytokine/Chemokine Magnetic Bead Panel Premixed 19-Plex; EMD Millipore, Billerica, MA USA

^b MAGPIX; Luminex Corporation, Austin TX USA

^c xPONENT v.4.2; Luminex Corporation, Austin TX, USA

^d MILLIPLEX ANALYST v.5.1, EMD Millipore, Billerica, MA USA

^e SAS v.9.4; Cary, NC USA

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**Chapter 4c. Evaluation of Serum Cytokines in Cats with and without Degenerative
Joint Disease and Associated Pain**

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Introduction

Degenerative joint disease (DJD) may be the most common disease in cats, with a prevalence of up to 92%, and increasing radiographic burden with increasing age.¹ Radiographic evidence of DJD is frequently accompanied by changes in mobility, activity, and social interactions,²⁻⁴ yet discrepancies exist between radiographic signs, orthopedic exam findings, and owner-reports of impairment.^{5,6} Treatment options for this pervasive disease are lacking, in part due to the difficulty in accurately assessing pain and associated disability in cats. There is a need for objective methods to measure DJD-associated pain in order to advance our understanding of the disease and our ability to develop effective treatments. Accelerometry has been useful as a surrogate measure of mobility and activity in cats,⁷⁻⁹ and advances in analytical methods for accelerometry data are being developed. However, these monitors remain costly and cumbersome, and offer no insight into the pathophysiology of the disease.

Development of biomarkers is a growing initiative in the fields of osteoarthritis (OA) and pain research in human and veterinary medicine.¹⁰⁻¹² Biomarkers, generally, may comprise any physical, imaging, or biochemical marker of disease or disease symptomology. Ideally, these markers would be sensitive to the presence of disease, indicative of the severity of disease, and responsive to treatments for the disease. In human DJD/OA research, soluble biomarkers of central interest include markers for structural damage to the joint and inflammation, both of which contribute to the clinical manifestation of pain. Inflammatory cytokines, particularly IL-1 β , IL-6, and TNF- α , have been implicated in the pathogenesis of pain and joint disease¹³⁻¹⁵, and elevated concentrations of these cytokines have been detected

in synovial fluid samples from human DJD/OA patients.¹⁶ Several chemokines, have also been demonstrated to be associated with chronic pain in both rodent models¹⁷ and human DJD/OA patients.^{10,18}

Despite their promise, the clinical heterogeneity of the disease in humans and the fluctuations in biomarker concentrations associated with intermittent flaring of symptoms have led to the suggestion that combinations of biomarkers, rather than a single universal marker, will be needed in the study of OA.¹⁹ This holds true across the category designations biomarkers are suggested to be useful for - Burden of disease, Diagnosis, Prognosis, Efficacy of intervention, and Investigative markers (BIPED).²⁰

In cats, despite the widespread nature of the disease, and the difficulty in measuring the impact of the disease, soluble biomarkers have not been investigated as indicators of DJD and associated pain. Suggestions from a recent proteomic and genomic study of cats with DJD found gene expression differences between cats with DJD and an age-matched group of cats without DJD were particularly evident in three main pathways: immune function, apoptosis, and oxidative phosphorylation.²¹ Proteomic analysis of serum found that cats with DJD had an increase in components of the complement system as well as down-regulation of the complement system regulator clusterin.²¹ Up-regulation of the complement system can lead to activation of macrophages and inflammatory cytokine secretion,²² and cartilage matrix components can activate complement.²³ Proteomic analysis of human synovial fluid also found increased expression of complement proteins in DJD/OA patients compared to individuals without OA.²³ It follows, therefore, that the pattern of cytokine expression may

be different in cats with DJD and associated pain compared to cats without DJD, and that this difference could potentially be detected in serum.

The development of multiplex technologies has allowed for the simultaneous quantification of multiple analytes using a very small volume (10-25 μ L) of sample. Recently, a feline-specific panel has been developed and validated that allows quantification of 19 cytokines and chemokines using multiplex technology^a. Given the associations between cytokines and painful DJD/OA in humans,^{10,17} this study was designed to use the feline-specific panel to evaluate concentrations of cytokines and chemokines in well-phenotyped cats with and without DJD. Furthermore, as a preponderance of cats with clinically evident DJD have concurrent chronic kidney disease (68% of cats in the study population in one report),²⁴ we sought to evaluate the interactive effect of pain and chronic kidney disease (CKD) status on cytokine concentrations. The effects of age and body condition were also investigated as age-associated inflammation is established in people²⁵ and obesity and increased adipokines have been implicated as risk factors for the development of DJDs.²⁶

Our central hypotheses were that cytokine/chemokine profiles in cats with DJD would differ from those of normal cats, and that these profiles could be used as surrogate measures of pain in cats with DJD, and in cats with DJD and concurrent CKD. Further, we hypothesized that combinations of cytokines/chemokines would be able to reliably distinguish between cats with painful DJD and normal cats.

Methods and Materials

Subjects and samples

Samples used in this study were banked serum samples that had been collected from cats presented to the Comparative Pain Research Laboratory over the period from May 2007 - May 2015. All cats had been examined by a veterinarian, and had been evaluated for systemic disease, orthopedic pain, and radiographic evidence of DJD as previously described.^{7,27,28} Briefly, all cats received a physical examination, including evaluation of body condition score (BCS),²⁹ followed by an orthopedic examination during which each joint or spinal segment was palpated and gently manipulated, and scored for the presence and severity of pain, crepitus, thickening, and effusion. Pain was scored on the following scale: 0 = no resentment; 1 = mild withdrawal, mild resistance to manipulation; 2 = moderate withdrawal, body tenses, may orient to site, may vocalize or increase vocalization; 3 = orients to site, forcible withdrawal from manipulation, may vocalize, hiss, or bite; and 4 = tries to escape or prevent manipulation, bites or hisses, marked guarding of site. Total pain (TPain) scores were calculated as the sum of the scores for individual joints, with a possible range of 0-80.

Following the physical and orthopedic examinations, cats were sedated using an individually tailored protocol and orthogonal digital radiographs were made of each joint and spinal segment. Radiographs were evaluated by a single investigator (BDXL) and scored for the presence and severity of DJD based on previously published criteria established by our laboratory.²⁷ Scores were ascribed according to a 10-point scale where 0 = no evidence of

DJD and 10 = ankylosis of the joint. Total radiographic DJD (TDJD) scores were calculated as the sum of the scores for individual joints, with a possible range of 0-200.

Prior to sedation, all cats had urine samples collected for urinalysis by cystocentesis, and blood samples collected for a complete blood count, serum biochemistry analysis, and for sample archiving. Whole blood collected for serum sample storage was collected into a sterile 3mL anti-coagulant free glass tube (red top Vacutainer®) and allowed to clot at room temperature for at least 30 but no more than 60 minutes. Clotted samples were centrifuged at 3400 rpm for 10 minutes, and serum was removed, aliquoted, and stored in cryovials at -80°C until use. Based on results from the serum biochemistry, urinalysis and review of radiographs and previous medical records, cats were classified as CKD positive or negative according to the guidelines set forth by the International Renal Interest Society.³⁰

Stored serum samples were eligible for inclusion in the study if they had been collected from cats that had been evaluated in the Comparative Pain Research Laboratory who had received a physical and orthopedic examinations, and radiographic evaluation of each joint and spinal segment. Further inclusion criteria included that the samples had been maintained at -80°C and had not been through more than 1 freeze-thaw cycle prior to testing.

Cytokine assays

Samples were analyzed for concentrations of 19-analytes using commercially available feline-specific multiplex cytokine kits^a. Kits were used according to manufacturer

recommendations, and samples were run in triplicate with all replicates from an individual cat run on the same plate. Quality control samples were run, in duplicate, on each plate.

Plates were analyzed using a dedicated plate reader and software^{b,c} and results were analyzed using statistical software packages^{d,e}. For each plate, quality control samples, standard curves, and bead counts were assessed according to manufacturer recommendations. Coefficients of variation (CVs) were evaluated for the results from each set of triplicates; CVs less than 20% were considered acceptable. If a set of replicates had a CV greater than or equal to 20%, individual results were examined. Despite limitations to this approach, if two replicates from a set of three were in close agreement, and the third was markedly different and driving the high CV, this third replicate was flagged and the results carried forward were based on the mean of the two remaining replicates. However, if none of the three replicates were in close agreement, results for that set of replicates were not included in any further analyses. Where CVs were acceptable, output results were based on the mean of all three replicates.

Statistical analysis

Descriptive data were tabulated to obtain distributions for age, sex, body condition score, TPain score, TDJD score, and CKD status among the cats. TPain scores and TDJD scores were evaluated as both continuous variables and as categorical variables according to the following designations (based on clinically relevant distribution of scores from the prevalence study of randomly selected cats across age groups).²⁷ TPain scores were categorized as 0-2=negligible/normal (as long as no single joint received a score of 2); 2-

4=low (a score of 2 was placed in this category if a single joint received a score of 2); 5-9=moderate; 10 and above=high. Given the difficulty in evaluating cats for pain, and unknown significance of low pain scores, analyte concentrations were compared between cats with pain scores in the negligible/normal and low categories using ANOVA. As no significant differences were found between these group for any analyte, groups were pooled into the normal/low group. This was clinically meaningful as cats in this combined category would be considered clinically unaffected by joint pain. TDJD scores were categorized as 0-3=negligible/normal; 4-12=low; 13-24=moderate; 25 and above=high. Again, given the unknown significance of low DJD scores, analyte concentrations were compared between cats with DJD scores in the negligible/normal and low categories using ANOVA, and were subsequently pooled for further analyses as the normal/low group, with the exception of one analysis (IL-18 analyte concentration as a function of DJD score category and CKD status) where a significant difference between the normal and low groups was found. Cats were also categorized as CKD negative or CKD positive (IRIS Stage 1 – 4, inclusive) as described.

Results where concentrations were below the lower limit of quantification (LLOQ) for a given analyte were imputed as equal to the $LLOQ/\sqrt{2}$,³¹ while results that were unable to be used (due to high CVs) were set as missing and designated NA. Analytes with greater than 50% of results below the LLOQ were excluded from the analyses. To address issues of fit, natural log transformation was applied to analyte concentration data for all analyses except correlations.

Correlations between the analyte concentration and TPain and TDJD scores were calculated for each cytokine. The effects of BCS, age, and sex on the natural log transformed analyte concentrations were modeled using ANOVA.

To explore relationships between pain, DJD, CKD, and analyte concentration, several analyses were performed. Natural log transformed analyte concentrations were modeled as functions of: 1. Pain category (3-level), CKD status (Pos/Neg), and the interaction of Pain category and CKD status; 2. Pain category (3-level), DJD category (3-level), and the interaction of Pain category and DJD level; and 4. TPain score, TDJD score, and CKD status (Pos/Neg).

In order to determine if a group of analytes would discriminate between cats with DJD and pain, and those without, discriminant analysis was used in the following comparisons: 1. Cats with normal and low pain/DJD versus cats with moderate and high pain/DJD; 2. Cats with normal and low pain versus those with moderate or high pain in the absence of DJD; and 3. Cats with normal and low DJD versus cats with moderate and high DJD in the absence of pain. Discriminant analysis was performed using step-wise selection to identify analytes for entry into the model. Once analytes were identified, canonical discriminant analysis was run using identified analytes to evaluate model fit, and plots were visually inspected for group separation.

Cluster analysis was performed to allow measured analyte concentrations to delineate groupings of cats, with the intent of identifying unifying features among the clusters.

Following clustering, a pseudo-T-squared plot was evaluated to determine the optimal number of clusters. Univariate analyses were used to evaluate the relationship between BCS, Age, TPain score, and TDJD score and cluster identification.

For all analyses, significance was set at $p < 0.05$. As these were exploratory analyses, correction for multiple testing was not performed, but comments on the significance given the number of tests performed is presented in the discussion. All assumptions for each test were evaluated, including assumptions of normality.

Results

Descriptive data for cat age, BCS, sex, and CKD status within each pain and DJD category are presented in Table 1. Pain score data from four cats are missing due to incomplete orthopedic exams (exams unable to be completed due to temperament). Data from these four cats were retained for analyses restricted to DJD scores and DJD score categories.

Table 1. Descriptive data for cats across the pain and DJD categories.

DJD Category	Pain Category	N	Age (Median)	Age (Min)	Age (Max)	BCS (Median)	BCS (Min)	BCS (Max)	CKD Status (#Positive)	Sex (Proportion Male)
Normal	Normal	11	4.32	1.41	18.30	5	1	6	0	0.55
	Low	5	3.43	1.04	5.75	5	4	7	2	0.60
	Moderate	2	4.30	2.57	6.03	7.5	7	8	0	1.00
	High	11	9.41	2.07	18.30	6	4	9	3	0.73
Low	Normal	3	5.74	4.92	9.91	4	4	7	1	0.00
	Low	4	10.36	2.05	17.02	5	1	5	3	0.75
	Moderate	5	13.49	10.32	15.55	7	4	7	3	0.80
	High	35	10.76	3.23	16.02	7	4	9	9	0.46
	Missing	1	18.31	18.31	18.31	7	7	7	1	1.00
Moderate	Low	3	11.27	8.31	12.63	7	5	7	0	0.67
	Moderate	5	10.39	5.01	19.27	7	3	8	2	0.60
	High	42	13.02	6.30	18.30	7	3	9	16	0.43
	Missing	2	10.22	9.02	11.41	5	4	6	1	0.00
High	Low	1	18.30	18.30	18.30	3	3	3	0	1.00
	Moderate	7	13.32	10.98	17.01	7	5	7	3	0.29
	High	48	13.48	4.76	19.89	7	1	9	20	0.42
	Missing	1	16.05	16.05	16.05	5	5	5	1	0.00

Assay results for each analyte were tabulated by the number and percent of samples that were above and below the LLOQ, and presented in Table 2. Fifteen of the 19 analytes had results above the LLOQ for at least 50% of the sample sets. Four analytes did not meet this criterion (FAS, GM-CSF, IL-1 β , and PDGF-BB) and were excluded from further analyses. The percentage of samples that were in range and below the LLOQ for each analyte

were tabulated by DJD score category and pain score category and are provided as Supplementary Table 1.

Table 2. Number of samples that were above and below the LLOQ, and the percentage of samples above the LLOQ are provided for each analyte. NA represents the number of sample sets with missing data. Analytes with >50% of samples above the LLOQ are in bold.

Analyte	Below LOQ or in range?				
	In Range	Low	NA	Total	% In Range
FAS	83	103	0	186	44.6%
FLT-3L	185	0	1	186	99.5%
GM-CSF	27	159	0	186	14.5%
IFN-γ	159	26	1	186	85.5%
IL-12P40	185	0	1	186	99.5%
IL-13	183	3	0	186	98.4%
IL-18	165	21	0	186	88.7%
IL-1 β	67	118	1	186	36.0%
IL-2	105	81	0	186	56.5%
IL-4	175	11	0	186	94.1%
IL-6	140	45	1	186	75.3%
IL-8	162	24	0	186	87.1%
CXCL-1	96	90	0	186	51.6%
MCP-1	179	6	1	186	96.2%
PDGF-BB	37	149	0	186	19.9%
RANTES	186	0	0	186	100.0%
SCF	185	0	1	186	99.5%
SDF-1	137	49	0	186	73.7%
TNF-α	96	90	0	186	51.6%

Correlations between analyte concentration and TPain and TDJD scores show few significant relationships

Results from correlations between untransformed analyte concentration and TPain and TDJD scores are shown in Table 3. Significant correlations were found between analyte concentration and TPain score for TNF- α and between analyte concentration and TDJD score for IL-4, however correlations for each of these results were considered low ($r=0.24$ and $r=0.14$, respectively).

Table 3. Correlations between analyte concentrations and TPain scores and TDJD scores. Significant correlations are in bold.

Analyte	Value	TPain Score	TDJD Score
FLT-3L	Correlation coefficient	0.13	0.049
	P-value	0.08	0.508
	N	181	185
IFN- γ	Correlation coefficient	-0.006	0.006
	P-value	0.934	0.938
	N	181	185
IL-12P40	Correlation coefficient	0.006	-0.088
	P-value	0.941	0.235
	N	181	185
IL-13	Correlation coefficient	0.138	0.098
	P-value	0.064	0.184
	N	182	186
IL-18	Correlation coefficient	0.007	-0.092
	P-value	0.927	0.214
	N	182	186
IL-2	Correlation coefficient	0.119	0.067
	P-value	0.109	0.365
	N	182	186
IL-4	Correlation coefficient	0.048	0.144
	P-value	0.516	0.049
	N	182	186
IL-6	Correlation coefficient	0.103	0.037
	P-value	0.166	0.614
	N	181	185
IL-8	Correlation coefficient	0.093	0.048
	P-value	0.214	0.517
	N	182	186
CXCL-1	Correlation coefficient	0.049	0.029
	P-value	0.515	0.696
	N	182	186
MCP-1	Correlation coefficient	-0.067	-0.075
	P-value	0.373	0.311
	N	181	185
RANTES	Correlation coefficient	0.119	0.097
	P-value	0.11	0.186
	N	182	186

Table 3 (continued).

SCF	Correlation coefficient	0.136	0.06
	P-value	0.069	0.419
	N	181	185
SDF-1	Correlation coefficient	0.005	0.03
	P-value	0.951	0.683
	N	182	186
TNF-α	Correlation coefficient	0.241	0.113
	P-value	0.001	0.123
	N	182	186

Body condition score, age, and sex are each significantly associated with individual analytes

Evaluation of the relationship between BCS, age, and sex with natural log transformed analyte concentrations found that overall model results were not significant for the majority of analytes tested, with the exception of IL-13 ($p < 0.001$), IL-2 ($p = 0.007$), IL-8 ($p = 0.006$), and CXCL-1 ($p = 0.045$). Specific results showed that BCS was significantly and positively associated with cytokine concentration for IL-13 and IL-2; age was significantly and positively associated with cytokine concentration for IL-8; and sex was significantly associated with cytokine concentration for CXCL-1 (concentrations in females were higher than males) (Table 4). The estimates (the pg/mL increase in cytokine concentration expected for every unit increase in parameter) was low for IL-8, even when considering age is typically measured in years.

Table 4. A. Specific results for analytes with significant overall model effects (IL-13, IL-2, IL-8, and CXCL-1).

	Parameter	Estimate	Standard Error	t Value	Pr > t
IL-13	BCS	0.162	0.042	3.82	<0.001
IL-2	BCS	0.149	0.057	2.62	0.009
IL-8	Age (days)	0.0002	0.0001	2.94	0.004
CXCL-1	Sex (FS)	0.486	0.207	2.35	0.020

Pain score category, but not CKD status, is associated with concentrations of IL-2 and IL-8

Overall model effects for the natural log transformed analyte concentrations and categorical pain score, CKD status, and the interaction between categorical pain score and CKD status showed significant effects for IL-8 ($p < 0.0001$) and MCP-1 ($p = 0.024$), and near-significance for IL-2 ($p = 0.052$). Significant effects were found for pain score category for IL-2 ($p = 0.027$) and IL-8 ($p = 0.006$), while no specific significant effects were found for MCP-1 (all $p > 0.05$). Least-squares means for IL-2 and IL-8 are shown in Table 5. For both IL-2 and IL-8, analyte concentration increased with worsening pain score category. Neither CKD status nor the interaction of pain score category and CKD status had a significant effect on analyte concentration.

Table 5. Least squares means results for IL-2 and IL-8 showing the relationship between pain score category and analyte concentration. Analyte concentrations for both IL-2 and IL-8 were significantly different (higher) between the high pain score category and the normal/low category, but not significantly different between normal/low and moderate, or between moderate and high.

Analyte	Pain Score Category	Log(Analyte) Least-Squares Mean	Standard Error
IL_2	Normal/Low	1.778	0.320
	Moderate	2.246	0.321
	High	2.659	0.124
IL-8	Normal/Low	2.131	0.276
	Moderate	2.409	0.278
	High	2.973	0.107

DJD score category and the interaction of DJD score category and pain score category are significantly associated with concentrations of IL-4 and IL-8

Overall model effects for transformed analyte concentrations and DJD score category, pain score category, and the interaction of DJD score category and pain score category found significance for IL-4 ($p=0.016$) and IL-8 ($p<0.001$). Significant effects were found for DJD category for IL-8 ($p=0.038$) and for the interaction of DJD category and pain score category for IL-4 ($p=0.020$) with near significance for DJD category ($p=0.056$). Tukey-Kramer post-hoc testing showed the same positive relationship for IL-8 concentration reported in the previous model, where concentrations were higher with worsening DJD score category, with a significant difference between the normal/low and the high pain categories. The interaction between DJD score category and pain score category and effect on analyte concentration was less clear for IL-4, with concentrations remaining fairly stable across DJD score categories for the high pain score group, increasing across DJD score categories for the moderate pain score group, and increasing and then decreasing across DJD score categories for the normal/low pain group (Table 6), however the only significant differences were between the

analyte concentrations in the high and normal/low DJD category within the moderate pain category.

Table 6. Results of post-hoc testing showing the relationship between analyte concentration and the interaction of pain score category and DJD score category for IL-4. Significant differences were found between moderate pain/normal/low DJD and moderate pain and high DJD, with non-significant differences between the remaining category combinations.

Analyte	Pain Category	DJD Category	Number	Mean	Std Error
IL-4	Normal/Low	Normal/Low	8	3.832	0.277
	Normal/Low	Moderate	9	4.625	0.766
	Normal/Low	High	7	3.336	1.328
	Moderate	Normal/Low	5	2.652	0.502
	Moderate	Moderate	6	4.260	0.594
	Moderate	High	4	5.330	0.502
	High	Normal/Low	2	4.293	0.196
	High	Moderate	3	4.408	0.205
	High	High	1	4.449	0.192

Increasing TDJD score and TPain score, but not CKD status, are associated with increased concentrations of IL-4, IL-8, and TNF- α .

We modeled the natural log of analyte concentrations as a function of TDJD score, TPain score, and CKD status, and found significant overall effects for IL-4 ($p=0.0431$), IL-8 ($p<0.0001$), and TNF- α ($p=0.022$). Post-hoc analysis showed that TDJD score was significantly and positively associated with both IL-4 and IL-8 concentrations, while TPain score was significantly and positively associated with both IL-8 and TNF- α concentration (Table 7). No significant effects were found for CKD status and any analyte.

Table 7. Results of post-hoc testing for analytes with significant overall model effects (IL-4, IL-8, and TNF- α).

Analyte	Parameter	Estimate	Standard Error	t-Value	Pr > t
IL-4	TDJD score	0.020	0.008	2.68	0.008
IL-8	TDJD score	0.015	0.007	2.30	0.023
	TPain score	0.049	0.013	3.66	<0.001
TNF- α	TPain score	0.051	0.022	2.33	0.021

Increased concentration of IL-8 in DJD and pain may be confounded by the association with age

The IL-8 results showed increased serum concentration with increases in DJD score and pain score, and an increased concentration with increased age. We ran two separate models with age as the response variable and pain score category or DJD score category as the explanatory variables; both models were highly significant ($p < 0.0001$) indicating that the relationships between pain score and age, and DJD score and age are too correlated to separate meaningfully. However, using a stepwise selection model allowing for DJD category, CKD status, pain category, age, and all the two-way interactions, only pain category remained in the model suggesting that increased pain score category explains more of the increase in IL-8 concentration than age.

Discriminant analysis was unable to identify one or more cytokines that distinguish between groups of cats classified based on DJD score category or pain score category

Three canonical discriminant models were run to determine if the results from one or more analytes could be used to distinguish between groups of cats based on their DJD score category or pain score category. Canonical discriminant analysis is a method that “finds

linear combinations of quantitative variables that provide maximal separation between classes or groups".³² Groups of cats undergoing comparison are shown in Table 8.

Table 8. Distribution of cats into categories for discriminant analyses. Four cats were excluded due to missing pain scores. Color blocks show groups compared within the series of analyses.

Table of Pain Category by DJD Category					
Pain Category	DJD Category				
	Normal	Low	Moderate	High	Total
Normal	11	3	0	0	14
Low	5	4	3	1	13
Moderate	2	5	5	7	18
High	11	35	42	48	137
Total	29	47	50	56	182

The first discriminant analysis comparison was between cats with no or low pain and no or low DJD (highlighted in yellow) and those with moderate and high pain and DJD (highlighted in dark green). Based on results from step-wise selection, the analytes selected for inclusion in the canonical discriminant analysis model were IL-8 (p=0.018) and FLT-3L (p = 0.024). The adjusted R² of this model was 0.026, and histograms show poor separation of the groups (Figure 1-A). The second comparison was to distinguish between cats with no or low pain (without DJD – highlighted in yellow) from those with moderate or high pain (without DJD – highlighted in light green). Analytes selected for inclusion in this canonical model were MCP-1 (p = 0.079), IL-8 (p = 0.049), IFN- γ (p = 0.072), and IL-13 (p = 0.043). Again, the adjusted R² of this model was quite low (0.037) and histograms showed poor separation of the groups (Figure 1-B). The final comparison was between cats with normal and low DJD versus cats with moderate and high DJD in the absence of pain. The only

analyte selected for inclusion in this model was IFN gamma ($p = 0.071$). The adjusted R^2 of this model was quite low (0.022) and histograms showed poor separation of the groups (Figure 1-C).

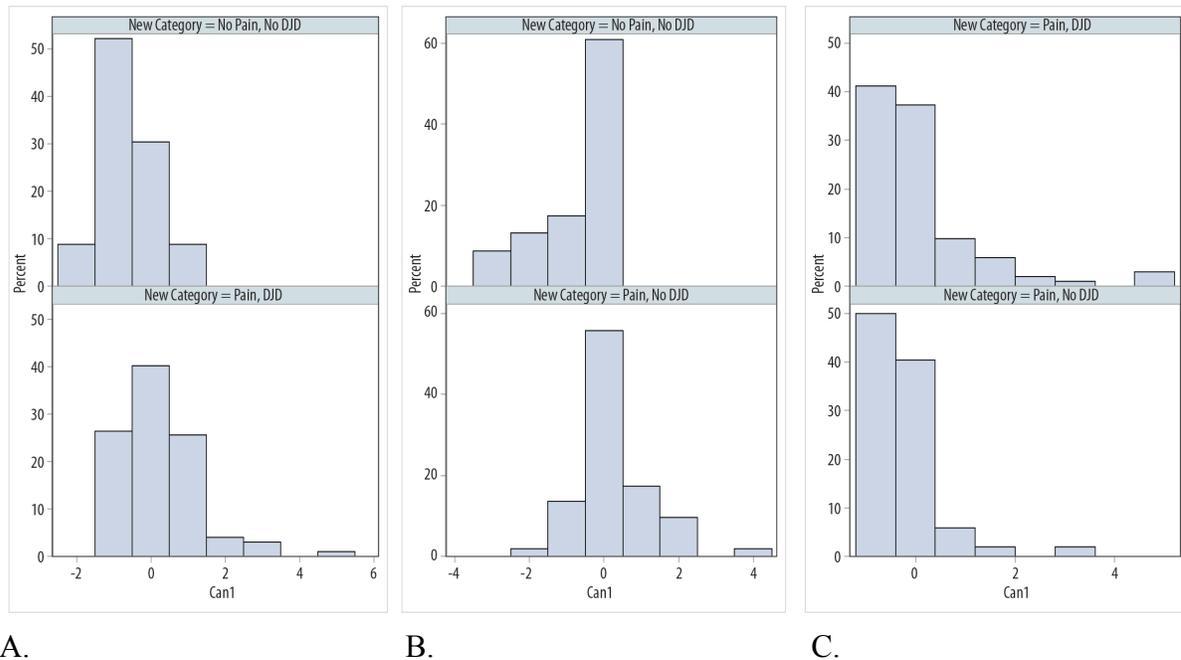


Figure 1. Histograms showing canonical discriminant analysis results for (A) cats with normal and low pain/DJD versus cats with moderate and high pain/DJD; (B) cats with normal and low pain versus those with moderate or high pain in the absence of DJD; and (C) cats with normal and low DJD versus cats with moderate and high DJD in the absence of pain. Poor separation of the groups is seen for all three analyses.

Cluster analysis driven by analyte concentrations show separation of groups of cats, but features defining the groups remain unknown

Cluster analysis was performed using the analyte concentrations to drive the clustering. Observation of the pseudo-T plot indicated that 4, 5, 9, 11, and 15 clusters were appropriate to use. Median values of 5 and 9 clusters were chosen, with 5 clusters ultimately providing reasonable separation of the groups (Figure 2) and number of cats per cluster.

Adjusted R^2 for this model with 5 clusters was 0.472, and all analytes except IL-8 ($p=0.302$)

and CXCL-1 ($p=0.333$) were significant for the clusters. Univariate and contingency analyses were run to determine the relationship between known variables and cluster identification, with no significant findings for age, BCS, TDJD score, and TPain score (all $p>0.05$).

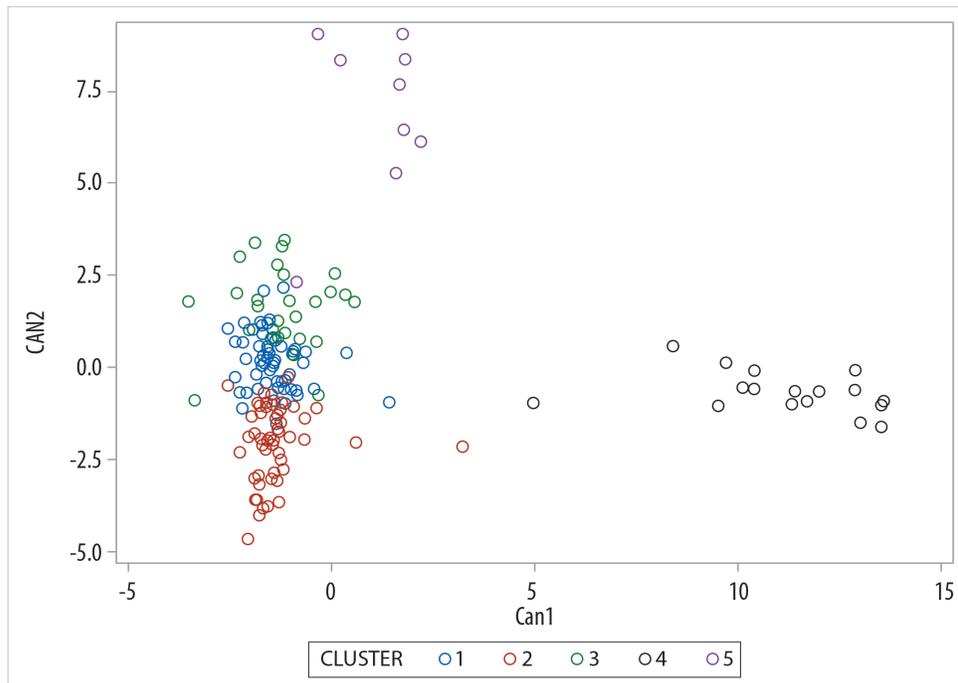


Figure 2. Results using five cluster showing improved separation of the groups of cats, particularly those in clusters four and five as compared to one, two, and three.

Discussion

This study showed that a small number of cytokines, IL-4, IL-8, IL-2, and TNF- α are associated with higher radiographic burdens of DJD and higher orthopedic pain scores. Specifically, we showed that cats with higher radiographic DJD scores have higher serum concentrations of IL-4 and IL-8, while cats with higher orthopedic exam pain scores have higher concentrations of IL-8, IL-2, and TNF- α . The association of increased IL-8

concentration with increasing pain score and DJD score was strong, but the confounding increase in IL-8 with increasing age encourages caution regarding conclusions about the importance of this finding. As there were not age matched control samples available for analysis, this finding may be more strongly associated with age than with DJD or pain, however pain score category appeared to explain more of the increase in IL-8 concentration than age in our model. A recent study has suggested that DJD in cats is associated with changes in genetic and proteomic profiles more extreme than simple aging.²¹

Based on our findings, we suggest that there are measureable changes in cytokine concentrations in cats with DJD that reflect true associations with DJD burden and pain. As normal ranges for cytokine concentrations in cats have not been determined, we cannot conclude that the increased concentrations found here are greater than normal, but only that they appear increased in cats with DJD and pain relative to normal cats. Nevertheless, our findings are supported by literature evaluating the associations between cytokines in DJDs in humans.^{14,33} There is growing interest in the interaction between nociceptors and the immune system, and the function that these interactions play in facilitating pain states.^{34,35} The role of TNF- α in nociceptor sensitization is well supported in the literature (reviewed in³⁶); it has been shown to target nociceptors both directly (via modulation of ion channels) and indirectly (through increased release of downstream cytokines). In addition, TNF-alpha is able to rapidly increase the firing of A- and C-fibers in the dorsal root ganglion, increasing transmission of input into the spinal cord.⁽³⁷ and reviewed in³⁸) Further, one study demonstrated a correlation between serum TNF- α concentration and pain scores in people with knee OA as measured by the Western Ontario and McMaster Universities Osteoarthritis

Index.¹⁰ There is growing interest and some demonstrated efficacy of targeted therapies against TNF- α in reducing joint pain,³⁹ though further studies are needed.

TNF- α is commonly mentioned in conjunction with IL-6 and IL-1 β , interleukins also capable of sensitizing nociceptors and frequently implicated in the pathogenesis of pain in DJD/OA.¹⁴ In our study, IL-1 β was only detected (above the lower limit of quantification) in 36% of the samples tested, and IL-6 was not associated with any of our measures of DJD or pain. The reason for this discrepancy between our findings and the literature base is unknown. While this could be a species-specific difference, it is more likely that it relates to the use of serum, as most studies of cytokines in DJD/OA have been conducted using *in vitro* preparations or synovial fluid. Still, IL-1 β ¹⁶ and IL-6¹⁵ have been detected in serum from patients with OA and our findings deserve further investigation. IL-2 and IL-8 have been less consistently associated with pain, though each has been investigated in experimental or chronic pain conditions. IL-2 has been demonstrated to have both hyperalgesic and analgesic properties in rodent models, dependent on location and route of administration.^{40,41} In one study, the concentration of IL-8 was found to be higher in plasma samples from OA patients than controls,⁴² while another study found higher levels of IL-8 mRNA expression from synovial biopsies in OA patients than patients with meniscal tears,⁴³ but other studies have failed to show a strong relationship between IL-8 and pain. It does appear that IL-8 is one of the cytokines downstream of TNF- α that may be produced by chondrocytes.⁴⁴ In the study reported here, IL-4 concentration was positively associated with radiographic DJD score. To our knowledge, this has not been demonstrated before, and merits further verification and potentially investigation. IL-4 is classically considered an anti-inflammatory cytokine, and

may be a marker of chronic inflammation, which could explain the increase seen here in cats with long-standing disease.

Cytokines are ubiquitous as they are involved in cell signaling throughout the body, and may be produced by multiple cell types. The cytokines found to be elevated in this study have many potential sources, including peripheral leukocytes or local sources such as monocytes/macrophages, synovial cells, or chondrocytes in the affected joints. While synovitis in degenerative elbow joints in cats has been shown to be relatively mild,⁴⁵ synovitis in OA in people is also typically scored as mild-moderate, and yet there is recognition of the inflammatory component to the disease.⁴⁶ Toll-like receptor 4 (TLR4) has been particularly implicated in contributing to inflammation in DJD/OA, with increased TLR4 expression in areas of damaged cartilage in patients with OA.⁴⁷ TLR4 signaling results in an increased inflammatory response and expression of several inflammatory mediators.⁴⁸ Cytokine production may also occur at the level of the dorsal root ganglion; astrocytes and microglia have been shown to release TNF- α and IL-6 in response to toll-like receptor-4 activation during inflammation,³⁸ and activated microglia in the dorsal root ganglia have been noted in rodent models of DJD/OA.⁴⁹ Glial activation has also been noted in human patients with chronic lower back pain.⁵⁰ Regardless of source, the increases demonstrated here, detected in the serum, likely indicate that low-level systemic inflammation is present in cats with DJD. The detrimental effects of even mild systemic inflammation on health are the focus of a growing area of research. Low-grade inflammation has been implicated in the development of several chronic disorders and central nervous system diseases including Alzheimer's disease.^{51,52} Other possible sequelae of systemic

inflammation include sickness behaviors, insulin resistance, diabetes mellitus, and autoimmune diseases. Cats are susceptible many of these, including an analog cognitive disorder (termed cognitive dysfunction syndrome) and the epidemiological links between these disorders and DJD deserves further research.

Despite the associations discovered, we were unable to determine a cytokine-associated signature of DJD in cats. In this study there was no single or group of cytokines that could reliably distinguish between cats with and without pain and DJD, cats with and without pain in the absence of DJD, or between DJD categories among cats with pain. There are several potential reasons for this, not the least of which is the remarkable complexity with which the balance of cytokines exists in the body, and the multiple sources of these cytokines. A recent study evaluated the ability of serum biomarkers to distinguish between groups of people with rheumatoid arthritis (RA), OA, and those free of disease using artificial neural networking. The resulting decision tree involved 15 nodes, with TNF- α at the top, and failed to group RA, OA, or normal patients together, highlighting the complexity of the interactions.⁵³ Other potential explanations overlap with limitations of the current study, notably that cats were examined and samples were taken at a single time point, while cytokine concentrations may change over time or over the course of the disease. Criteria for staging DJD in cats have not yet been developed, and are hindered by lack of understanding of the critical features of worsening clinical disease, but it is possible that sub-populations of DJD phenotypes in cats exist and are associated with different inflammatory profiles.

The use of serum samples, rather than synovial fluid, has been mentioned previously as another potential limitation in our ability to detect differences between groups of cats. Cytokines and chemokines are typically produced and consumed locally, and detection of increased concentrations in peripheral blood would likely reflect much higher concentrations at the sites of release. An additional limitation in the use of serum is the contribution to cytokine load from comorbid conditions including age,²⁵ obesity,⁵⁴ and chronic kidney disease.⁵⁵ While we evaluated the relationship between measured cytokine concentrations and age, body condition, and the presence or absence of chronic kidney disease and found few positive associations, each likely contributes to the overall circulating concentration of cytokines in these cats. In DJD research, the majority of soluble biomarker work has been performed using synovial fluid samples as these are reflective of the microenvironment within the damaged joint. Unfortunately, synovial fluid is difficult to obtain from cats, even from degenerative joints where a low to moderate amount of effusion is present. The banked serum samples in our lab, from well-phenotyped cats, were a reasonable starting point for this work, as the use of serum is more clinically applicable, and findings from serum could be compared to findings in synovial fluid from a smaller number of cats. Future work using synovial fluid samples from degenerative and normal joints of cats should be performed.

Additional limitations include potential systematic measurement error for TPain scores, as cats were evaluated by one of three separate investigators. Rigorous training of investigators by the lead veterinarian (BDXL) mitigated the impact of this, and all investigators involved in the studies were licensed veterinarians with clinical experience with cats. No such limitation is expected regarding DJD scores as a single investigator (BDXL)

completed all DJD scoring based on previously published criteria developed by our laboratory.

The noted limitations were motivation for our second approach to cluster analysis, allowing the concentrations of the cytokines to drive the clustering of the cats. This analysis led to better separation of the groups of cats, and the majority of the cytokines used in the analysis were significantly associated with the clustering. However, the common features among cats in a given cluster are yet to be determined. The clustering of the cats does not appear to relate to age, body condition, DJD or pain. Whether these clusters are unrelated to the presence or absence of pain, or represent a limitation of our phenotyping that can be addressed remains to be ascertained.

Another potential limitation of the current study is the age of some of the samples, as these were collected over years of work with cats by our lab. Stability of cytokines in serum has been studied in human samples, but no work has yet been done in cats. Based on the literature using human samples, cytokine concentrations can decrease over years of storage, even when held in -80°C ,^{56,57} and it is reasonable to expect that some degradation could have occurred. As older samples were not systematically different from other, more recently collected samples (i.e., not all normal cat samples were older), the likely impact of sample age would be on decreasing our ability to detect a difference between groups. This must be balanced by a reduction in power if those samples were excluded. Samples included in this study were controlled for freeze-thaw cycle, as concentrations of cytokines have been shown to change (both increase and decrease) in response to repeated freeze-thaws. Again, this has

not been systematically evaluated in serum samples from cats, but initial work by the manufacturer of the cytokine panel has shown that this is a potential confounder for cytokines in cats (personal communication) if multiple freeze-thaw cycles occur. Finally, the use of the commercially available panel artificially focused our study to the provided analytes. Other soluble biomarkers of interest, based on findings in other species, include markers of cartilage degradation such as cartilage oligomeric protein or matrix metalloproteinases in serum, or CTX-II in urine (reviewed in ²⁰). However, the panel was chosen for discovery work due, in part, to the paucity of feline-specific assays and availability of the technology. Despite its limitations, the 19 analytes provided by the panel include many of those of interest in DJD/OA and pain, and mark the first investigation of these analytes in a population of cats with and without DJD and pain. Further, the multiplex platform allows for screening of multiple analytes using a very small volume of sample, and specific findings from this study can be followed with targeted ELISAs, particularly to verify both the increases found and the absence of increases in IL-1 β and IL-6.

In conclusion, we found that there are measurable increases in cytokines that are associated with increased burden of DJD and increased pain in cats relative to cats without DJD and pain. Despite the inability of one or more cytokines to reliably discriminate between groups of cats with and without DJD and pain, this work contributes to the burgeoning field investigating the interactions between the immune system and DJD/OA associated pain. Further work to verify these findings, and extend investigation into the sources of the increased cytokines will help elucidate the mechanisms underlying the associations found

here. These findings also support the inclusion of cats in the important translational work on naturally-occurring arthritides, and the need for development of feline-specific assays.

^a FCYTMAG-20K-PMX Feline Cytokine/Chemokine Magnetic Bead Panel Premixed 19-Plex; EMD Millipore, Billerica, MA USA

^b MAGPIX; Luminex Corporation, Austin TX USA

^c xPONENT v.4.2; Luminex Corporation, Austin TX, USA

^d MILLIPLEX ANALYST v.5.1, EMD Millipore, Billerica, MA USA

^e SAS v.9.4; Cary, NC USA

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Supplemental Table 1. Percent of samples that were in range or below the LLOQ for each analyte by DJD score category and Pain score category. Distributions that were significantly different by category (Fisher's Exact test, $p < 0.05$) are in bold.

Analyte	DJD category	In Range (%)	Low (%)	Pain category	In Range (%)	Low (%)
FAS	Low/normal	37.7	62.3	Low/normal	25.9	74.1
	Moderate	51.9	48.1	Moderate	52.6	47.4
	High	47.4	52.6	High	47.8	52.2
FLT-3L	Low/normal	98.7	0.0	Low/normal	100.0	0.0
	Moderate	100	0.0	Moderate	100.0	0.0
	High	100	0.0	High	99.3	0.7
GM-CSF	Low/normal	13.0	87.0	Low/normal	3.7	96.3
	Moderate	19.2	80.8	Moderate	10.5	89.5
	High	12.3	87.7	High	16.9	83.1
IFN- γ	Low/normal	80.5	18.2	Low/normal	74.1	25.9
	Moderate	84.6	15.4	Moderate	74.7	26.3
	High	93.0	7.0	High	89.7	9.6
IL-12P40	Low/normal	98.7	1.3	Low/normal	100.0	0.0
	Moderate	100.0	0.0	Moderate	100.0	0.0
	High	100.0	0.0	High	99.3	0.7
IL-13	Low/normal	98.7	1.3	Low/normal	96.3	3.7
	Moderate	96.1	3.8	Moderate	100.0	0.0
	High	100.0	0.0	High	98.5	1.5
IL-18	Low/normal	87.0	13.0	Low/normal	88.9	11.1
	Moderate	92.3	7.7	Moderate	89.5	10.5
	High	87.7	12.3	High	88.2	11.8
IL-1 β	Low/normal	29.9	68.8	Low/normal	18.5	81.5
	Moderate	46.1	53.8	Moderate	26.3	73.7
	High	35.1	64.9	High	41.2	58.1
IL-2	Low/normal	57.1	42.9	Low/normal	44.4	55.6
	Moderate	63.5	36.5	Moderate	47.4	52.6
	High	49.1	50.9	High	61.0	39.0
IL-4	Low/normal	92.2	7.8	Low/normal	88.9	11.1
	Moderate	6.1	3.8	Moderate	94.7	5.3
	High	94.7	5.3	High	95.6	4.4
IL-6	Low/normal	68.8	29.9	Low/normal	59.3	24.2
	Moderate	82.7	17.3	Moderate	63.2	36.8
	High	77.2	22.8	High	80.1	19.1
IL-8	Low/normal	75.3	24.7	Low/normal	66.7	33.3
	Moderate	92.3	7.7	Moderate	73.7	26.3
	High	98.2	1.7	High	93.4	6.6

Supplemental Table 1 (continued).

CXCL-1	Low/normal	45.4	30.6	Low/normal	37.0	63.0
	Moderate	51.9	28.0	Moderate	52.6	47.4
	High	59.6	40.3	High	54.4	45.6
MCP-1	Low/normal	92.2	6.5	Low/normal	85.2	14.8
	Moderate	98.1	1.9	Moderate	94.7	5.3
	High	100.0	0.0	High	99.3	0.0
PDGF-BB	Low/normal	18.2	81.8	Low/normal	14.8	85.2
	Moderate	21.1	78.9	Moderate	10.5	89.5
	High	21.1	78.9	High	22.1	77.9
RANTES	Low/normal	100.0	0.0	Low/normal	100.0	0.0
	Moderate	100.0	0.0	Moderate	100.0	0.0
	High	100.0	0.0	High	100.0	0.0
SCF	Low/normal	98.7	1.3	Low/normal	100.0	0.0
	Moderate	100.0	0.0	Moderate	100.0	0.0
	High	100.0	0.0	High	99.3	0.7
SDF-1	Low/normal	68.8	31.2	Low/normal	51.8	48.2
	Moderate	75.0	25.0	Moderate	78.9	21.1
	High	78.9	21.1	High	76.5	23.5
TNF- α	Low/normal	46.7	53.2	Low/normal	44.4	55.6
	Moderate	61.5	38.5	Moderate	47.4	52.6
	High	49.1	50.9	High	54.4	45.6

Chapter 5 : Conclusions

This dissertation set out to address several unmet needs in the field of research on degenerative joint disease (DJD) and associated pain in cats – unmet needs that should be addressed if we are to advance the treatment of pain associated with DJD in this species, and if we are to exploit any potential benefit this spontaneous disease model may have in translational research.

DJD has a remarkably high prevalence in cats; across all age groups, nearly 92% may have radiographic evidence of disease, with approximately half of those having evidence of associated pain and mobility impairment. Given this high prevalence, it is surprising that research into this disease is in its relative infancy, with the first systematic studies taking place only in the past decade. This is due, in part, to a lack of awareness of DJD on the part of owners and veterinarians, which, in turn, is due to species-specific differences in clinical presentation of DJD among cats. A significant proportion of the lack of awareness has likely been due to our inability to measure DJD-associated pain and pain relief. Growing interest in treatment options for DJD and associated pain is driving forward research in this field.

Further, there is burgeoning recognition that cats and dogs with DJDs represent important translational models of arthritides in humans, heightening the importance of better characterizing the behavioral and biological manifestations of DJD in cats. The work presented in this dissertation advances the field by furthering our knowledge of both the qualitative and quantitative assessment of pain associated with DJD. This was accomplished through understanding features of study design and subjective outcome measures, including the caregiver placebo effect (qualitative) as well as deepening our understanding of the

activity patterns of cats with and without DJD and investigation of inflammatory cytokine concentrations in cats with and without DJD (quantitative).

Recruitment and participation in clinical trials

Surveys of cat owners and veterinarians

We began with the most fundamental of the issues – access to cats and their owners for clinical and translational research studies. In clinical trials run through our laboratory, we have faced issues related to recruitment of suitable cases in a timely fashion. While the issue of recruitment into clinical trials is well researched and much discussed in human medicine, research in veterinary medicine has been completely lacking.

We began with a survey of cat owners to determine features of trial design and protocols that would influence their participation in a clinical trial with their cats. Our results were quite similar to findings in pediatric human medicine where a proxy caregiver is often responsible for the decision to participate in a trial. We found that owners were most likely to participate if their cat had a disease that would benefit from the trial, and they trusted the organization performing the trial. The highest rated incentives for participation included free veterinary services and financial incentives (gift cards).

We also found that the majority of cat owners rated their veterinarian's recommendation of the trial as important (24%) or extremely important (56%) in considering participation in a trial. This led us to our follow-up survey of veterinarians, where we found that, while very few veterinarians are engaged in clinical research, more had investigated and

even recommended a clinical trial to a patient. Trial features that were favorable for clinician recommendation included low invasiveness, safety of the treatment, respect for the investigator as a researcher, and belief in the value of the study. Many veterinarians indicated that engagement in the study via dissemination of the results would make them more likely to recommend a clinical trial. In general, awareness of available clinical trials could be improved among veterinarians, and opportunities for easy access and engagement, including on-line databases, would be valuable.

Caregiver placebo effect and mitigation

Quantification of the effect

Next, we set out to quantify the caregiver placebo effect in trials of analgesics for cats with DJD, an effect that has stymied development and evaluation of potential therapeutics, and to develop relevant mitigation strategies for this effect. Using available data from the majority of placebo-controlled studies performed with client-owned cats with DJD, we found that the caregiver placebo effect was remarkably high, with 68% of owners rating cats as improved on subjective outcome measures while cats were receiving a placebo.

Intriguingly, 23% of these cats also improved on objectively measured activity, which could be an effect of the natural waxing and waning course of the disease, or could indicate that a change in interaction between owners and cats occurs when owners believe their cats to be on an active treatment. This change in interaction could lead to an increase in activity for the cat (a placebo-by-proxy effect). A caregiver placebo effect this high makes it quite difficult for potential therapeutics to show efficacy over a placebo.

Refinement of outcome measures and study design

We explored refinements to existing outcome measures, including the use of a percent of possible score for the Feline Musculoskeletal Pain Index developed by our group, and evaluation of a novel study design. The most fascinating finding was the novel use of deterioration, which is a return of clinical signs following cessation of an active treatment, as a measure of efficacy against a placebo. In a double-blinded trial, we found that many owners that had given their cats a placebo during the first period of a clinical trial rated their cats as improved, and that this rating remained stable during the subsequent period when continued on placebo. In contrast, owners that had given their cats an active treatment during the first period also rated their cats as improved, but during the subsequent placebo period, their cats' clinical signs returned and their ratings deteriorated, with separation of the groups by the end of this period. This study design, using a return of clinical signs as a measure of efficacy for a therapeutic, must be evaluated in larger trials, but has the potential to alter how analgesic therapies are tested in cats.

Development and refinement of objective measures

Functional data analysis

Finally, we turned to development and refinements of objective measures. First, we applied the relatively new method of functional data analysis (FDA) to data from accelerometers worn by cats. Accelerometry, or activity monitoring, is currently the most widely used objective outcome measure in cats with DJD. Previous studies have focused on summary values obtained by averaging activity counts per epoch (typically 1-minute) across days or weeks. While this approach has been useful and convenient, it requires the loss of

information about the pattern of activity in cats – information that could provide insight into both impairment and improvement in cats with DJD and associated changes in activity.

Using FDA, we were able to show that differences exist in the pattern of activity shown during a 24-hour day by cats with DJD as compared to those without DJD, and that this pattern was significantly affected by age and orthopedic pain score. Further, we demonstrated that cats in homes exhibit a bimodal pattern of activity, with different patterns on weekends and weekdays. Further work to apply FDA to the analysis of treatment effects for analgesics is underway.

Evaluation of cytokines

Second, in an effort to advance the field of biomarker development, we measured serum cytokine and chemokine concentrations in well-phenotyped cats with and without DJD and associated pain using a multiplex panel of 19 different cytokine/chemokines. We found that individual analytes, in particular IL-4, IL-8, IL-2, and TNF- α were associated with radiographic DJD score and orthopedic pain score. The strongest findings were for IL-8, where increased concentrations of IL-8 were associated with increased DJD score and increased pain score, although, the association of increased IL-8 concentration with increased age and the increase in both DJD score and pain score that occur with age may confound this relationship. While no single or group of analytes was able to reliably differentiate cats with and without DJD, this investigation was the first of its kind to assess the potential for a soluble biomarker associated with DJD or DJD-associated pain in cats and lays a foundation for further work with soluble biomarkers.

Anticipated impacts

Given the prevalence of DJD in cats, the debilitating nature of the disease, and the vast potential for advances in the study of DJD in cats to benefit both cat and human health, further work in this field remains a critical need in veterinary medicine.

These future studies will benefit from our studies. Through the work presented in this dissertation, we have moved forward our understanding of how to design, recruit for, and assess outcomes in studies of DJD in cats. These are critical to furthering advancement of therapeutics for this debilitating disease, and for development of DJD in cats as a model for the study of DJD/OA in humans. We now have insight into why cat owners would participate in clinical trials with their cats, and what features of clinical trials can influence veterinarian recommendations. We have developed a study design that has great promise for the evaluation of analgesics in veterinary medicine, and we have identified an exciting opportunity to determine the effectiveness of this design in studies that use different formulations and dosing intervals. Return of clinical signs as a measure of efficacy has potential applications beyond pain studies, and extension of this design into other disciplines may yield a powerful study design tool, particularly in studies of chronic conditions that rely on proxy measures with subjective scales, such as behavior and dermatology. The application of functional data analysis to accelerometer data allows us to understand the pattern of activity in cats, and future work will evaluate the effect of analgesics in shifting this pattern. As cats are potentially a better model of spontaneous activity, evaluating the patterns of activity and understanding how these are affected is crucial for translational research. As a result of this dissertation, work is being done using detailed owner diaries to better

understand the influence of human patterns of activity and interaction on cat activity in the home.

Much remains to be done. While educational efforts aimed at veterinarians are improving awareness among practitioners, we need fuller understanding of owners' recognition of DJD in their cats, and the educational interventions that can impact this. Collaboration with experts in communication could be valuable in testing the utility of various educational materials, including handouts and videos of cats with DJD and mobility impairment, and also social media outreach. Greater consumer awareness will increase the demand for a viable therapeutic, and create an environment where developed outcome measures can be applied in a wider arena. It will also increase the demand for feline-specific assays to deepen our understanding of the mechanisms underlying DJD and associated pain in cats, including longitudinal studies to better inform risk factors and temporal relationships between DJD and potential risk factors such as obesity.

Finally, further work is needed to understand the features motivating the caregiver placebo effect, as there are likely both experimental factors and owner-specific factors that influence this effect. We need to understand what the placebo represents in these studies, both to mitigate its effect in clinical trials as well as to enhance its effect in clinical practice.

Appendices

Appendix 1: Pathophysiology of pain in degenerative joint disease

Degenerative joint diseases (DJD), in particular osteoarthritis (OA), are common in humans, and pain is the dominant complaint. Pain associated with DJD appears to be driven by both peripheral sensitization of nociceptors and plasticity in the pain-processing pathway that contributes to an overall sensitization. Both peripheral and central processes resulting in sensitization can promote widespread (distant from the affected joint) hyperalgesia and allodynia, and shape the picture of clinical pain. Pain associated with DJD is a major cause of disability and functional/mobility impairment, yet understanding of pain in DJD remains incompletely understood.

Pain is the conscious perception of noxious input, and the sensation of pain is the result of transmission of this noxious input into the central nervous system by way of the dorsal horn of the spinal cord, or (for facial pain) the trigeminal ganglion, and projection of this input through various regions to the somatosensory cortex where the 'perception of pain' is encoded. Noxious stimuli are detected by nociceptors, (also called noci-receptive neurons) a group of afferent nerve fibers specialized for the detection of noxious stimuli. These nerve fibers can detect noxious thermal, mechanical, chemical, and electrical stimuli and then transduce information about these stimuli into electrical signals (nerve impulses) that are transmitted to the spinal cord and the brain. Nociceptors are widely distributed throughout the body including in the skin, muscles, dura, viscera, and joints.

Nociceptors include C- and A δ -fibers, and can be classified based on their dynamic range (the area they receive signals from), activation threshold, diameter, myelin thickness, type of stimuli they respond to, or type of sensation they produce. C-fibers are the smallest diameter, unmyelinated sensory afferents that typically have a wider receptive range than A δ -fibers, but are associated with a less discrete (more difficult to localize) sensation. C-fibers are the most numerous and widely distributed of the nociceptors, and are located cutaneously as well as in deeper, visceral organs. C-fibers are considered polymodal as they respond to noxious thermal, mechanical, or chemical stimulation, and C-fiber activation produces a dull, burning or aching pain sensation. A δ -fibers are also small to medium diameter, but (except for their terminal ends) are lightly myelinated leading to more rapid conduction than C-fibers. A δ -fibers respond to thermal and mechanical stimuli and produce a sharp, pricking pain. In addition to C- and A δ -fibers, A β -fibers also carry afferent sensory information from the periphery. A β fibers are large and thickly myelinated, with rapid conduction velocity. These fibers typically transmit non-noxious sensory information, and are responsible for the sensation of light touch, but in pathological pain states, A β -fibers may be involved in pain sensation.^{1,2}

The responses to noxious stimuli are mediated by receptors (transducers) present on the terminals of nociceptors. These include a mixture of ionotropic and metabotropic receptors, many of which have been identified, though several remain unknown. One prominent receptor type is the transient receptor family, including transient receptor potential vanilloid receptor -1 (TRPV1) implicated in sensitivity to noxious heat. In general, nociceptors will express a set of receptors (that defines the neuron phenotype), and will

typically exhibit a fairly high threshold for response. In the presence of injury or inflammation, this phenotype can shift as surface receptor expression is altered and thresholds for response are lowered. The source of this shift is linked to the release of sensitizing molecules, chiefly cytokines, chemokines, prostaglandins, bradykinin, and growth factors such as nerve growth factor (NGF) released in response to tissue injury or inflammation.³ These molecules can directly sensitize nociceptor receptors (through phosphorylation). These molecules also stimulate an inflammatory cascade resulting in infiltration of leukocytes into the area. Under normal circumstances and in response to injury, this is advantageous, encouraging protection of the area and healing. However, when the pain is persistent, long-term changes can take place in the nociceptor receptor profiles, such as NGF stimulated increased expression of TRPV1 receptors,⁴ that allow for lowered thresholds of activation. Additional sequelae seen are a widening of the receptive field of neurons, and involvement of A β fibers in nociception.³ These effects are recognized clinically as hyperalgesia (an exaggerated response to a noxious stimulus) within the area of injury or disease.

The primary sensory afferents (A- and C-fibers) have cell bodies in the dorsal root ganglion, and project to the dorsal horn of the spinal cord. Once these afferents enter the spinal cord, they form synapses with second order neurons, which may be inhibitory interneurons, or projection neurons. Projection neurons decussate and continue toward the brain, predominantly following the spinothalamic tract, terminating in the thalamus and synapsing with third order neurons that may project to the somatosensory cortex to effect pain perception. Alternatively, they may follow pathways that include those related to the

emotional aspects of pain and fear (via the limbic system), or contribute to pain modulation and descending inhibitory control of pain (Figure 1).

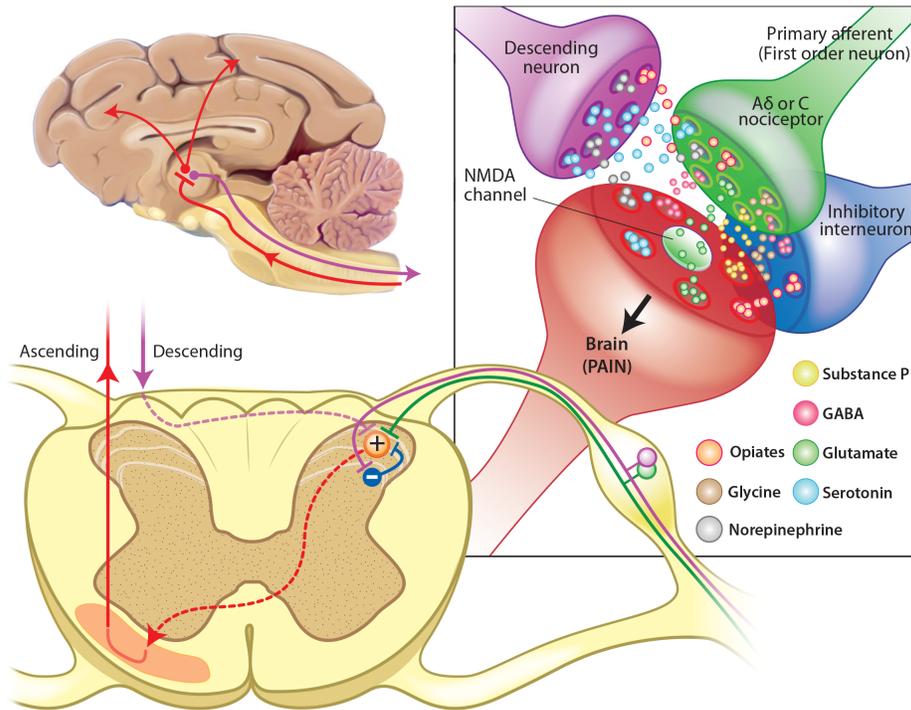


Figure 1. Schematic representation of ascending and descending pain pathways.

Endogenous modulation of pain can serve to facilitate or dampen the pain-signaling pathway, and this modulation may occur at the level of the dorsal root ganglion or higher brain regions. At the level of the dorsal root ganglion, local inhibitory interneurons modulate the transmission of nociceptive input. In the gate theory, proposed by Melzak and Wall in 1965,⁵ activation of projection neurons in the pain pathway depends on the balance of input from nociceptive and non-nociceptive sources. In the example shown in Figure 2, input from the non-nociceptive A β -fiber excites the inhibitory interneuron, while nociceptive input from

the C-fiber inhibits the inhibitory interneuron, and it is this balance that results in inhibition or disinhibition of the projection neuron. Additional modulation comes from descending pathways, particularly via the periaqueductal gray feeding through the rostroventral medulla (RVM), a system that involves endogenous opioids, endocannabinoids, serotonergic and noradrenergic systems.^{6,7} Cognitive processes may also modulate pain, as attention to or distraction from pain may decrease reported pain scores⁸.

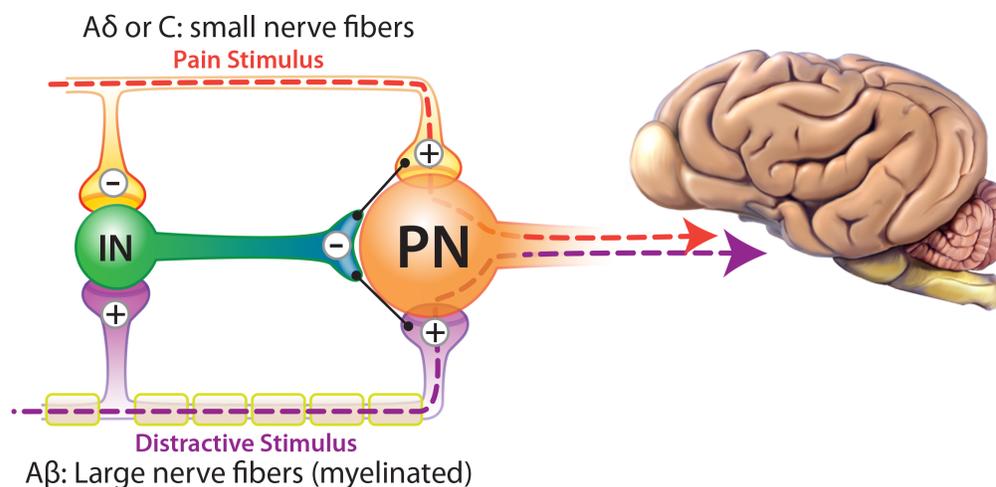


Figure 2. Gate theory of pain.

With persistent input from the periphery, particularly in states of persistent peripheral sensitization as in chronic diseases, changes in pain transmission and modulation occur in the spinal cord that result in central sensitization. Additionally, the persistent nociceptive input results in a concurrent decrease in the ability for the endogenous analgesic systems (from the RVM) to moderate pain. Central sensitization is initiated when nociceptive input into the spinal cord, results in the removal of the magnesium block on N-methyl D-aspartate (NMDA) receptors and resulting activation of NMDA receptors.² The ensuing NMDA-

mediated cellular wind-up leads to modulation of neuronal function through channel phosphorylation, activation of glia and microglia with release of cytokines and prostaglandins locally,⁹ and finally modification of gene induction and eventually connectivity changes. In addition to the local facilitation of nociceptive processing, there is a decrease in the effectiveness of the local inhibitory pathways.² Presumably as a result of increased nociceptive traffic to higher centers, there is a net decrease in the ability of the endogenous descending analgesic system. Collectively, this results in a net gain in the nociceptive transmission and perception of pain, and progressive alterations in the somatosensory system resulting in widespread pain, and is a characteristic of several chronic pain states, including DJD.² Such maladaptive changes in the central nervous system result in secondary hyperalgesia and allodynia.

In DJD and OA, pain is the dominant complaint of patients, yet understanding of the source of this pain is incomplete. Degradation of the cartilage is a hallmark feature of DJD/OA, but there are concurrent degenerative changes in all components of the joint - the synovium, periostium, and subchondral bone.¹⁰ While the cartilage is (at least initially) aneural and avascular, there is rich sensory innervation of the synovium, periosteum, subchondral bone, and surrounding musculature, all potential sources of nociceptive input in DJD. Typically, primary nociceptive afferents in the joint are unresponsive within the normal working range of the joint, but sensitization may occur in DJD such that normal movements are perceived as painful. Changes in central pain modulation and central sensitization are found in many patients with DJD/OA, with resulting lowered mechanical thresholds both at affected joints and remote sites.¹¹ The characteristic features of central sensitization in

DJD/OA are the presence of pain in regions remote from the affected joint, allodynia, and hyperalgesia, frequently assessed through quantitative sensory threshold testing. Central sensitization may become the driver of perceived pain, representing an obstacle to effective treatment with conventional therapies.¹²

For many years, DJD/OA was considered primarily a non-inflammatory, ‘wear and tear’ disease, with obesity and age regarded as risk factors for the increased mechanical load on the joints over time. This non-inflammatory etiology was endorsed as synovitis was generally considered mild to moderate, and synovial fluid cell counts are generally low.¹⁰ Recently, there has been a shift toward recognition of and research into the inflammatory component of DJD/OA, with evidence that the persistent, though low-grade, inflammation leads to changes in sensitivity and pain perception in patients.¹³ Beyond simple mechanics, ageing and obesity are appreciated as contributing to the increased inflammatory load.^{14,15} Studies of cytokine profiles in synovial fluid from DJD/OA patients report increased concentrations of several cytokines and chemokines that may be the result of activation of synovial macrophages,¹⁶ and these increased cytokine concentrations can contribute to the clinical signs of pain and disability via their roles in peripheral and central sensitization. Recently, evidence has shown that damage-associated molecular proteins (DAMPs), and advanced glycation end products (AGEs – a form of DAMPs associated with age-related DJD/OA) can upregulate Toll-like receptor 4 expression in chondrocytes, and increase production of interleukin-6 and COX-2.¹⁷ The role of Toll-like receptor 4 signaling in DJD/OA is a current topic of research in the field.¹⁸

Contributing to the lack of understanding of the sources of pain in DJD is the mismatch between measurable degeneration (via imaging) and patient self-report of pain. While factors such as bone marrow lesions¹⁹ and joint effusion²⁰ have been shown to be associated with higher reported pain, the relationship is far from perfect. This is due in part to the subjective nature of the pain experience, and the multi-dimensionality of pain, which draws not only on the physical sensation of pain, but also on the cognitive, emotional, and affective aspects of pain. Fear of pain and learned avoidance of pain triggers are features of DJD-associated pain for many patients, and can be as debilitating as the pain itself.²¹ Tempering these may be social support, coping mechanisms, and personality traits such as dispositional optimism.²² A ‘neuromatrix’ approach to pain attempts to understand the contributions from these many facets on the clinical picture of chronic pain.

Treatments for DJD may be palliative, addressing the pain associated with DJD, and/or disease modifying. Palliative treatments are typically analgesics and anti-inflammatories, and non-steroidal anti-inflammatory drugs (NSAIDs) are the current mainstay of therapy. As a class, NSAIDs act to block production of prostaglandins through inhibition of cyclooxygenase (COX) isoforms 1 and/or 2 (Figure 3), thereby decreasing prostaglandin E₂-mediated inflammation and neuronal sensitization both in the periphery and in the central nervous system.¹² However, by inhibiting COX 1/2, NSAIDs also prevent the production of prostacyclins and thromboxanes, leading to some of the associated adverse drug reactions seen with chronic NSAID therapy.²³ In the past several years, new formulations of NSAIDs have been available for clinical use, typically with greater COX-2 selectivity, but safety profiles may not be dramatically improved.

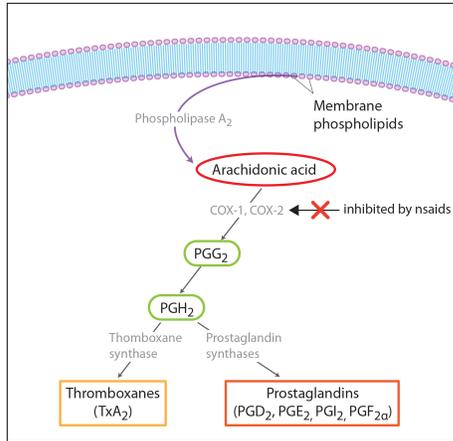


Figure 3. Production of prostaglandin from membrane phospholipids and the site of inhibition by NSAIDs.

Other therapeutics currently in clinical use for treatment of DJD-associated pain include opioids, serotonin and norepinephrine reuptake inhibitors (SNRIs), gabapentin, and NMDA-receptor antagonists. Novel therapies are in development, with a focus on targeted therapies based on existing understanding of the mechanisms of pain, including piperantans which block one receptor of PGE₂, the EP₄ receptor, and also antibodies directed at NGF.

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Appendix 2: VP Survey

Thank you for participating in this survey study. The following questionnaire is aimed at veterinary professionals and is designed to provide input on views on clinical research in veterinary medicine. **This study is for research purposes only and is not involved with commercial or marketing interests.** Our objective is to better understand factors involved in veterinarians' thoughts and attitudes toward clinical research. Your participation is greatly appreciated.

For the purposes of this survey, a clinical research trial is a study that enrolls patients for the evaluation of a procedure, medication, or device. These may be controlled evaluations of existing modalities, or evaluation of new or experimental procedures, medications, or devices. The ultimate aim of these studies is benefit to the veterinary patient population.

We estimate the questionnaire will take 10-15 minutes to complete. The identity of individual responses will remain anonymous in data analysis and no personal details will be collected or passed on to third parties. Please complete the questionnaire only one time.

PART 1

- * 1. Are you a ...? Please select all options that apply:
- Veterinarian
 - Nurse/Technician
 - Veterinary Student
 - Student Nurse/Technician
 - Practice manager
 - Other – please specify
- * 2. Are you a member/affiliate of ...? Please select all options that apply:
- VIN
 - AAFP
 - ISFM
 - BSAVA
 - AVMA
 - AAHA
 - None
 - Other – please specify
3. For qualified (graduated) veterinarians, please provide:
- a. The institution and country awarding your vet degree
 - b. Your year of graduation
- * 4. Where is your current practice/employment located? Please enter your work place postal or ZIP code
- * 5. In which country are you currently employed?
- * 6. Which term best describes your current practice/employment?
- General practice (small animal only, +/- exotics)
 - General practice (mixed animal)
 - General practice (large animal only)
 - Exotics only
 - Feline only
 - Referral/specialty
 - Research/Academia
 - Industry
 - Shelter/rescue/charity clinic
 - Other, please specify
7. Please select all the species you see in your practice/employment:
- Dogs
 - Cats
 - Small mammals
 - Avian species
 - Reptiles
 - Equine & Food Animal
 - Small ruminants
 - Zoo/large exotic species
 - None
- a. Over the past year, what percentage of your caseload has been dogs?
 - b. Over the past year, what percentage of your caseload has been cats?
 - c. Over the past year, what percentage of your caseload has been small mammals?
 - d. Over the past year, what percentage of your caseload has been avian species?
 - e. Over the past year, what percentage of your caseload has been reptiles?
- * 8. Do you spend some percentage of your time engaged in research?
- Yes
 - No

PART 2

- * 9. Have you recommended a clinical trial for one of your clients/patients in the past?
- Yes
 - No
- * a. In the future, would you consider recommending a clinical trial for one of your patients?
- Yes
 - No
 - Unsure
- * 10. Have you participated in a clinical trial in the past (screened or enrolled patients, collected data, etc)?
- Yes
 - No
- * 11. What areas, if any, within veterinary medicine do you think are in the most need of randomized clinical trials?
- * 12. How do you find information about clinical trials in your area?
- Word of mouth
 - Mailshot/mailed flyers
 - Emailed flyers/adverts
 - Through workplace
 - Through journals
 - Clinical trial website
 - I do not typically hear about clinical trials in my area
 - Other, please specify

PART 3

* Please indicate your level of agreement or disagreement with the following statements (strongly disagree to strongly agree)

13. I believe that clinical trials are important in veterinary medicine
14. I believe that current therapies (already in use) should be evaluated by clinical trials rather than based on anecdotal evidence
15. I believe that I received enough education on clinical trials during veterinary school
16. I feel comfortable discussing clinical trials and informed consent with my clients
17. I believe veterinarians have a role in recommending clinical trials to owners
18. I believe veterinarians should select the clients/patients they recommend for clinical trials rather than promoting information about all available trials
19. I believe pet owners in my area are aware of clinical trials that are available
20. I believe most pet owners at my practice would be fearful about clinical trials with their pet

PART 4

* Please indicate whether the following factors would make you more, or less likely to recommend a clinical trial to one of your patients (most likely to least likely):

21. The clinical trial would be conducted within my practice/hospital
22. The clinical trial was sponsored/run by an academic institution
23. The clinical trial involved an investigator I was familiar with (and/or thought highly of)
24. The clinical trial was sponsored by industry
25. Results of the clinical trial would be made available to me
26. Results of the clinical trial would be published, even if findings were negative
27. The trial involved a new or experimental medicine or procedure
28. The trial compensated me for my time/involvement
29. The trial compensated owners/pets for their involvement
30. The trial involved a medication or procedure approved in another country but not yet in my country
31. The trial involved collection of routine samples that could be collected non-invasively (such as fecal samples, hair samples, wearing a small, external monitoring device)
32. The trial involved collection of routine samples that could be collected in a minimally invasive way (such as venipuncture for blood samples, urinalysis)
33. The trial involved collection of non-routine samples that required more invasive sampling (such as endoscopy or biopsy)
34. The trial was testing a treatment for a disease that was uncommon/rare
35. The trial was testing a new treatment for a disease with an established, successful standard of care
36. Please list any other influential factors that we did not ask about (optional):

PART 5

* Please rate the importance to you in considering recommendation of clinical trials
(1= Not at all important and 5 = Extremely important)

- 37. Inconvenience to the owner
- 38. Concern about putting the research study needs and protocol before patient needs
- 39. Concern about changes to the Doctor-Patient-Client relationship
- 40. Fear of loss of trust from owners/clients
- 41. Concern about loss of case control
- 42. The amount of time required to explain the trial to the owner (including risks/benefits)
- 43. Belief in the importance of the study
- 44. The amount of time required to assess/treat/enroll the patient
- 45. Concern about loss of clients
- 46. The amount of paperwork that would be required by the trial
- 47. Concern about my understanding or knowledge of available clinical trials
- 48. Please list any other important factors that we did not ask about (optional):

* 49. How much time, per case, would you be willing to dedicate to a clinical trial (case recruitment, sample collection, etc.)?

- None
- 1-15 min/case
- 16-30 min/case
- 31-45 min/case
- 46-60 min/case
- >1 hour/case

* 50. Considering only dogs and cats, and a study lasting 4 months, how many visits do you think the average DOG owner would be willing to make to participate in a trial?

- More than one visit per week
- One visit per week
- One visit per month
- One visit every other month
- One visit total

* 51. Considering only dogs and cats, and a study lasting 4 months, how many visits do you think the average CAT owner would be willing to make to participate in a trial?

- More than one visit per week
- One visit per week
- One visit per month
- One visit every other month
- One visit total

PART 6

This is the second last section... nearly there!

* Please indicate whether the following owner/pet characteristics would make you more or less likely to recommend a clinical trial (much less likely to much more likely)

- 52. Owner lived in a remote location
- 53. Owner was economically disadvantaged
- 54. Owner lacked reliable transportation
- 55. Owner did not have English as a first language
- 56. I believed the patient would be cooperative
- 57. The pet was healthy
- 58. The pet was sick, but would likely recover
- 59. The pet had a very poor prognosis
- 60. There was a 20% chance of the pet receiving a placebo
- 61. There was a 50% chance of the pet receiving a placebo
- 62. There was an 80% chance of the pet receiving a placebo
- 63. Please list any other influential factors that we did not ask about (optional):

PART 7

Just one more section to go, thank you!

* Please rate what you believe is the importance, in general, to OWNERS in considering participation in a clinical trial (1 = Not at all important and 5 = Extremely important)

64. Their pet suffered from a condition that might benefit from the trial
65. Their veterinarian recommended the trial
66. The medication or procedure was experimental or unproven
67. Distance they would need to travel for the trial
68. Number of visits required for the trial
69. Length of each visit required for the trial
70. Their pet would need to wear a monitoring device of some type
71. They would be required to complete a daily journal for the trial
72. The trial required them to fast their pet before visits
73. The trial required 15-30 minutes each day (in activities, journaling, etc)
74. The trial required 30-45 minutes each day (in activities, journaling, etc)
75. Safety of the procedure/device/treatment under study
76. Costs associated with participating in the trial
77. Please list any other factors you believe are important to owners that we did not ask about (optional):

Final Questions

- * 78. How would you rate your overall feelings about clinical trials in veterinary medicine?
- Strongly negative
 - Negative
 - Neutral
 - Positive
 - Strongly positive
- * 79. What do you think is the best way to advertise clinical trials in veterinary medicine in your area?
- Email to the practice
 - Fax to the practice
 - Printed information sent to the practice
 - Visit from the study investigator to the practice
 - A clinical trials website for the area
 - Via continuing professional development/continuing education course (eg webinar, conferences)
 - Other, please specify
- * b. What do you think is the next best way to advertise clinical trials in veterinary medicine in your area
- Email to the practice
 - Fax to the practice
 - Printed information sent to the practice
 - Visit from the study investigator to the practice
 - A clinical trials website for the area
 - Via continuing professional development/continuing education course (eg webinar, conferences)
 - None
 - Other, please specify
- * 80. What do you believe would be the best incentive for owners considering participation in a clinical trial?
- Free services (labwork, radiographs, examinations)
 - Financial remuneration (such as gift cards)
 - Gifts for their pet
 - Other, please specify
- * b. What do you believe would be the next best incentive?
- Free services (labwork, radiographs, examinations)
 - Financial remuneration (such as gift cards)
 - Gifts for their pet
 - None
 - Other, please specify
81. Any other comments you would like to leave about clinical trials in veterinary medicine?

Appendix 3: VIN Survey

Q1. Are you currently practicing as a veterinarian who predominantly deals with canine and/or feline patients?

Q2. What best describes your current practice or employment position?

- General practice (small animal +/- exotics)
- General practice (mixed animal)
- Feline only
- Referral/specialty
- Clinical academia
- Shelter/rescue/charity clinic
- Mobile
- Relief
- Other
- Emergency medicine

Q3. Where did you obtain your veterinary degree?

Q4. When did you graduate from veterinary college?

Q5. In which country is your practice located?

Q6. How much of your time do you currently spend engaged in clinical research?

- None
- Less than 5%
- 5 to 10%
- 11 to 20%
- 21 to 50%
- More than 50%

Q7. Have you ever investigated enrolling a patient in a clinical trial?

- Yes
- No (if No, go to Q12)

Q8. How often do you investigate enrolling a patient into a clinical trial?

- Daily
- At least weekly
- At least monthly
- At least quarterly
- At least semi-annually
- At least yearly
- Less than once a year

Q9. Have you ever recommended enrolling a patient in a clinical trial?

- Yes
- No (if No, go to Q14)

Q10. In the last year, how often did you recommend enrolling a patient into a clinical trial?

- Daily
- At least weekly
- At least monthly
- At least quarterly
- At least semi-annually
- At least yearly
- I did not recommend it in the last year

Q11. Have you ever successfully enrolled a patient in a clinical trial?

- Yes (if Yes, go to Q17)
- No (if No, go to Q16)

- Q12. Why have you NOT investigated enrolling patients into any clinical trials? (Go to Q13)
- I am not interested in participating in clinical trials
 - My clients are not interested in participating in clinical trials
 - There are no clinical trials for the diseases or patients I deal with
 - Clinical trial centers are too far away from my practice
 - I don't want to lose the client to the study investigators
 - I don't have time to participate in clinical trials
 - Against current practice policy
 - Other

- Q13. How likely are you to consider investigating enrolling patients into a clinical study in the future? (Go to Q46)
- Very likely
 - Very unlikely
 - Somewhat likely
 - Somewhat unlikely

- Q14. Why have you NOT recommended enrolling patients into any clinical trials? (Go to Q15)
- I am not interested in participating in clinical trials
 - My clients are not interested in participating in clinical trials
 - There are no clinical trials for the diseases or patients I deal with
 - Clinical trial centers are too far away from my practice
 - I don't want to lose the client to the study investigators
 - I don't have time to participate in clinical trials
 - Against current practice policy
 - Other

- Q15. How likely are you to consider recommending enrolling patients into a clinical trial in the future? (Go to Q46)
- Very likely
 - Very unlikely
 - Somewhat likely
 - Somewhat unlikely

- Q16. Why have you NOT successfully enrolled patients into any clinical trials?
- I am not interested in participating in clinical trials
 - My clients are not interested in participating in clinical trials
 - There are no clinical trials for the diseases or patients I deal with
 - Clinical trial centers are too far away from my practice
 - I don't want to lose the client to the study investigators
 - I don't have time to participate in clinical trials
 - Against current practice policy
 - Other

- Q17. In the last year, how often did you successfully enroll a patient into a clinical trial?
- Daily
 - At least weekly
 - At least monthly
 - At least quarterly
 - At least semi-annually
 - At least yearly
 - I failed to enroll any cases in the last year

Please indicate the level of agreement with the following: (Strongly agree to strongly disagree)

- Q18. Veterinary clinical trials are important in advancing veterinary medicine
- Q19. Anecdotal evidence is sufficient for most therapies
- Q20. I feel comfortable discussing clinical trials and informed consent with my clients
- Q21. Veterinarians should recommend clinical trials to owners as often as possible
- Q22. Veterinarians should recommend clinical trials ONLY to select clients
- Q23. Most of my clients are aware of veterinary clinical trials
- Q24. Most of my clients are fearful about participating in a veterinary clinical trial

How likely would you be to recommend a clinical trial to a client based upon the following EXPERIMENTAL factors? (Extremely likely to Extremely unlikely)

- Q25. Trial examines novel therapy
- Q26. Trial examines anecdotal therapy
- Q27. Trial involves therapy licensed elsewhere
- Q29. Trial involves surgical intervention
- Q30. Trial involves a rare disease
- Q31. Trial examines new treatment for disease with currently successful therapy

How likely would you be to recommend a clinical trial to a client based upon the following METHODOLOGICAL factors? (Extremely likely to Extremely unlikely)

- Q32. Trial is non-invasive
- Q33. Trial is minimally invasive
- Q34. Trial involves invasive sampling procedures
- Q35. Data collection interferes minimally with daily activities
- Q36. Data collection interferes moderately with daily activities

How likely would you be to recommend a clinical trial to a client based upon the following ADDITIONAL factors? (Extremely likely to Extremely unlikely)

- Q37. You retain management of the case
- Q38. Trial is sponsored by an academic institution
- Q39. Trial is sponsored by a corporation
- Q40. Trial is not funded
- Q41. You were compensated for time
- Q42. Client was compensated
- Q43. Trial is run by a respected investigator
- Q44. You would be informed of trial results
- Q45. Trial results guaranteed to be published

How important do you consider the following factors in **your** decision to recommend a clinical trial to a client? (5 Point Scale)

- Q46. Inconvenience to the owner
- Q47. Concern about putting the research study needs and protocol before pet's needs
- Q48. Concern about my relationship with my client
- Q49. Loss of trust from client
- Q50. Loss of case control
- Q51. Time required to explain the trial to client
- Q52. Belief in the value of the study
- Q53. Time required to assess/treat/enroll the pet
- Q54. Paperwork that would be required by the trial

How important do you believe the following factors are to the CLIENT when they consider enrolling their pet in a clinical trial? (5 Point Scale)

Q55. Pet has a disease that could benefit from the trial

Q56. Veterinarian's endorsement

Q57. Treatment is experimental

Q58. Distance to trial center

Q59. Number of re-checks required

Q60. Duration of each recheck

Q61. Need to wear a monitor

Q62. Need to keep a daily log

Q63. Need to fast prior to recheck

Q64. Safety of the treatment

Q65. Cost of participation

Q66. Pet in placebo group

Q67. Free evaluation and treatment