Measurement of Arthritic and Bone Serum Metabolites in Arthritic, Non-Arthritic, and Geriatric Cats Fed Wellness Foods

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ABSTRACT

The objective of these studies was to evaluate arthritic markers and to determine the effects of feeding wellness-type foods on cartilage protection in geriatric cats. Study 1 utilized 26 cats to determine biochemical differences between arthritic and non-arthritic cats. Study 2 utilized 40 geriatric cats (>12 years of age) in an experiment to evaluate 4 age-appropriate wellness foods (experimental food, foods A, B, and C) for cartilage protection. All foods were fed to groups of 10 cats (40 cats total) for a period of 90 days.

In Study 1, osteocalcin and bone-specific alkaline phosphatase were significantly higher in the non-arthritic cats ($P = 0.06$ and $P = 0.04$, respectively). Carboxy-terminal linked telopeptide (ICTP) was significantly higher for the arthritic cats ($P = 0.03$). No differences were observed between the arthritic and non-arthritic cats for the other markers measured. In Study 2, the experimental food had significantly lower amino-terminal linked telopeptide (NTX) at Day 30 when compared with foods B and C ($P < 0.01$ and $P = 0.05$, respectively). The experimental food had significantly lower ICTP when compared with food B ($P < 0.01$). At Day 90, the experimental food had significantly lower NTX when compared with food B ($P < 0.01$) and food C ($P = 0.06$). At Day 90, the experimental food had significantly lower ICTP when compared with food B ($P = 0.02$). No differences were observed when comparing the experimental food to foods A, B, and C for pyridinoline at Day 30 or Day 90.

These data indicate that wellness foods can be developed without the use of synthetic glucosamine or chondroitin to protect the cartilage in the cat. The study also supports the use of multiple arthritic markers because of the high variability in individual markers alone.

INTRODUCTION

Osteoarthritis (OA) is a complex disease process of articular cartilage which is associated with a variable degree of synovitis from natural aging, trauma, and/or disease.[1] Osteoarthritis in cats is often not diagnosed in the early onset of the disease because of the cat's ability to accommodate to an orthopedic abnormality by redistributing the weight-bearing force to other limbs.[2] Additionally, because cats with OA are

more averse to climb or jump and are typically less active, many mild to moderate lamenesses due to OA go unnoticed by the cat's owner.[2,3]

Osteoarthritis can occur in cats at any age but is often diagnosed in geriatric cats (>12 years of age)[2] Hardie[2] studied the prevalence of OA in cats greater than 12 years of age and found that 20% of the cats (n = 68) had radiographic evidence of OA. Documented causes of OA in cats include developmental/traumatic conditions that can alter joint stability (ie, joint dysplasias), nutrient imbalances (ie, hypervitaminosis A and hyperglycemia), and neuropathic (diabetes mellitus) causes.[3-5]

Because early signs of feline OA typically remain elusive, identifying indications of OA via serum or urinary biomarkers would be a huge advantage to the veterinarian. Serum and urinary biomarkers have been studied as a means to assess cartilage damage in dogs under experimental conditions and to assess joint problems in clinical scenarios.[6-9] Unfortunately, little research is available on feline arthritis and no research is available for identifying potential biomarkers for OA. The understanding of the differences in biomarkers for collagen degradation and joint tissue metabolism in cats with or without visual signs of lameness would be useful in predicting joint metabolism prior to the onset or the advancement of the disease. This information could be used to generate technologies that target alterations in cartilage metabolism to either rebuild cartilage or to prevent cartilage damage in cats.

Nutritional technologies (ie, n-3 fatty acids and glucosamine/chondroitin [GAG]) have been studied and developed to overcome OA conditions in adult cats.[10] An alternative approach would be to change the propensity for arthritis by altering joint tissue metabolism prior to the onset of arthritic conditions. In order to develop intervention schemes, the basic physiological differences need to be established to determine the intervention points that can be manipulated by nutrition.

More information is needed on the effects of nutrition in the prevention of arthritis in cats. Therefore, the objectives of these studies are: 1) to determine which arthritic markers can be utilized to determine onset of arthritis and 2) to evaluate the effects of feeding a wellness-type food rich in n-3 fatty acids, methionine, and manganese with no synthetic GAG in the prevention or delay of onset of arthritic symptoms in healthy senior animals.

MATERIALS AND METHODS

Study 1

A total of 26 cats (13 with signs of naturally occurring lameness and 13 without signs of lameness) were identified for the study. The cats were cared for in accordance with Institutional Animal Care and Use Committee protocols. Radiographs were taken of the joint with positive visual signs for degenerative joint disease. The radiographs were scored as 0, 1, 2, or 3 based on the scoring system used by Hardie et al.[3]

Blood was drawn and collected, and the serum harvested and stored in 1-mL aliquots. The serum was analyzed for the following biomarkers using commercially available kits: carboxy-terminal cross-linked telopeptide (ICTP), amino-terminal cross-linked telopeptide (NTX), osteocalcin, bone-specific alkaline phosphatase (BAP), pyridinoline (Pyr), and total D-pyridinoline (D-Pyr) (Table 1).

Study 2

Cats and Treatments

The study utilized 10 healthy geriatric mixed-breed cats (≥10 years of age) per treatment group (40 cats total). The cats were cared for in accordance with Institutional Animal Care and Use Committee protocols. The cats were determined to be healthy by physical exam and blood chemistry screen. The study design utilized a 30-day pre-feeding period followed by a 3-month test feeding period for a total of 4 months.

Table 1. Arthritic and Bone Markers Measured in Blood in Study 1.

Metabolite	Non-Arthritic Cats	Arthritic Cats	Non-Arthritic vs Arthritic*
Osteocalcin, ng/mL	36.56	12.52	0.06
Bone specific alkaline phosphatase, ng/mL	6.35	3.43	0.04
Amino-terminal linked telopeptide, nM BCE	11.91	10.26	0.40
Carboxy-terminal linked telopeptide, ng/mL	8.38	13.05	0.03
Pyridinoline, nM/L	3.53	3.47	0.92
D-pyridinoline, nM/L	3.76	4.42	0.07

*Probability of greater F value.

During the 30-day pre-feeding period, all cats were fed a dry control food that was formulated in accordance with the Association of American Feed Control Officials (AAFCO)[11] nutrient guide for cats and balanced to meet maintenance requirements. During the last week of the pre-feeding period, blood samples were taken from each animal. Dual-energy x-ray absorptiometry (DXA) scans and Tekscan® pressure map analysis were also performed. The cats were then blocked by age, gender, and body fat percentage and assigned to 4 different treatment groups. Each group of cats was randomly assigned to receive either the experimental food or 1 of 3 commercially available age appropriate (10+ years of age) products.

The experimental food was formulated in accordance with the AAFCO[11] nutrient guide for cats and balanced to meet maintenance requirements. The 3 commercial foods included IAMS Active Maturity (Food A), Royal Canin Mature 28 (Food B), and Purina ONE Senior Protection Formula (Food C). The nutrient composition of each food is presented in Table 2. Cats were fed each food to maintain their weight throughout the duration of the study. Additionally, cats were offered enrichment toys, received routine grooming, and had daily opportunities for socialization with other cats and people.

Sampling

Blood samples were collected on Days 0, 30, and 90 of the test period. Serum was harvested and stored in 1-mL aliquots and later analyzed for fatty acids, arthritic markers, and bone markers. Cats were scanned by DXA at Day 0 and Day 90 to document changes in body composition and bone density. In addition, at Day 45 (Day 15 on treatment) and Day 120 (Day 90 on treatment), the cats were walked across the Tekscan® pressure map to determine effects of the foods on joint health via pressure vertical force (PVF). This procedure involves having the cats walk freely across a thin mat placed on the floor. The thin mat contained a thin-film tactile pressure/force sensor that produces accurate and reliable pressure and force readings for each step the animal takes. The mat is connected to a computer that captures the data, and the software shows real-time 3D and 2D color displays of the force exerted from each foot as it steps on the mat. The information is used to compare changes in the forces exerted by each step and then correlated to joint health and arthritis.[12,13]

Analysis of Blood Samples

Amino-terminal cross-linked telopeptide was determined using a commercially available ELISA (Wampole Laboratories, Princeton, NJ; Cat#9021). Bone-specific alkaline phosphatase, total D-Pyr, and Pyr,

Table 2. Analyzed Nutrient Profiles of the 4 Foods Utilized in Study 2.

Nutrients, 100% Dry Matter Basis	Control Food*	Experimental Food†	Commercial Food A‡	Commercial Food B§	Commercial Food C¶
Crude protein, %	36.13	35.73	34.85	30.52	40.45
Fat, %	29.77	22.47	15.39	23.63	15.69
Calcium, %	0.99	0.94	1.22	0.80	1.38
Phosphorus, %	0.74	0.77	1.05	0.72	1.30
EPA, %	<0.01	0.32	0.07	0.13	0.07
DHA, %	0.01	0.23	0.08	0.11	0.07
Linoleic acid, %	4.06	5.05	2.78	4.78	2.17
Total n-3 fatty acids, %	0.27	1.14	0.28	0.74	0.32
Total n-6 fatty acids, %	4.16	5.09	2.87	5.02	2.13
Methionine, %	0.88	1.32	1.05	0.72	0.77
Manganese, ppm	20	104	63	70	73

*Control food ingredient list: Poultry meal, corn gluten meal, rice, corn meal, animal fat, soy mill run, pal enhancer, cellulose, potassium chloride, choline chloride, calcium carbonate, calcium sulfate, DL-methionine, yeast, salt, potassium citrate, taurine, preservative, vitamin premix, mineral premix, and arginine.
†Experimental food ingredient list: Corn meal, poultry meal, corn gluten meal, animal fat, soybean mill run, soybean oil, beet pulp, fish oil, pal enhancer, calcium sulfate, potassium chloride, DL-methionine, choline chloride, L-carnitine, vitamin E, yeast, potassium citrate, vitamin premix, L-lysine, taurine, iodized salt, L-cysteine, mineral premix, L-threonine, and manganese sulfate
‡IAMS Active Maturity.
§Royal Canin Mature 28.
¶Purina ONE Senior Protection Formula.

were determined using commercially available ELISA (Quidel, San Diego, Calif; Cat#8012, Cat#8030, and Cat#8019, respectively). Osteocalcin was determined using a commercially available ELISA (Nordic Bioscience Diagnostics, Herlev, Hovedgade; Cat#3OSC4000). Carboxy-terminal cross-linked telopeptide was determined using a commercially available ELISA (Orion Diagnostica, Denmark; Cat#OD-06099). All fatty acids were analyzed using a modified method described by Rodriguez-Palmero et al.[14]

Statistics

Data were analyzed using General Linear Models procedure of SAS[15] to determine treatment means. The experimental unit was cat. In Study 2, Day 0 was used as a covariate. The experimental food was then compared with the 3 commercially available foods. Differences were considered significant when $P < 0.05$ and trends were determined when $P < 0.10$.

RESULTS

Study 1

Twenty six cats were radiographed to confirm the presence of arthritis and used to determine which markers would be applicable in Study 2. Osteocalcin and BAP were significantly higher in the non-arthritic cats ($P = 0.06$ and $P = 0.04$, respectively). Carboxy-terminal linked telopeptide was significantly higher for the arthritic cats ($P = 0.03$). No differences were observed between the arthritic and non-arthritic cats for NTX, D-Pyr, or Pyr.

Study 2

All cats remained healthy throughout the duration of the experiment. Serum fatty acids and arthritic markers measured in the blood at Day 30 are presented in Table 3. At Day 30, the cats fed the experimental food had significantly lower NTX when compared with foods B and C ($P < 0.01$ and $P = 0.05$, respectively). No differences were

Table 3. Serum Fatty Acids and Arthritic Markers Measured in the Blood in Cats at Day 30 Fed 4 Different Foods in Study 2.

Metabolite	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	Vs A*	Vs B*	Vs C*
NTX, nM BCE	12.0	12.1	15.4	13.6	0.93	<0.01	0.05
Pyr, nM/L	2.83	3.11	3.16	3.06	0.28	0.22	0.38
ICTP, µg/L	6.90	6.30	9.41	5.76	0.52	<0.01	0.22
EPA, mg/dL	5.3	1.5	3.4	2.3	<0.01	<0.01	<0.01
DHA, mg/dL	6.1	4.2	3.7	4.4	<0.01	<0.01	<0.01
Total n-6 fatty acids, mg/dL	56.3	55.5	68.1	47.1	0.83	<0.01	0.02
Total n-3 fatty acids, mg/dL	12.5	6.3	8.2	7.4	<0.01	<0.01	<0.01

*Probability of greater F value.

Table 4. Arthritic Markers and Serum Fatty Acids and Arthritic Markers Measured in the Blood in Cats at Day 90 Fed 4 Different Foods in Study 2.

Metabolite	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	Vs A*	Vs B*	Vs C*
NTX, nM BCE	17.4	18.2	22.8	19.8	0.49	<0.01	0.06
Pyr, nM/L	2.90	2.98	2.89	3.04	0.67	0.96	0.45
ICTP, µg/L	6.75	7.66	8.95	7.27	0.31	0.02	0.56
EPA, mg/dL	5.4	1.9	3.5	2.8	<0.01	<0.01	<0.01
DHA, mg/dL	6.5	4.7	4.4	5.1	<0.01	<0.01	<0.01
Total n-6 fatty acids, mg/dL	50.1	54.2	65.5	43.6	0.24	<0.01	0.07
Total n-3 fatty acids, mg/dL	13.0	7.3	9.1	8.7	<0.01	<0.01	<0.01

*Probability of greater F value.

observed when comparing the cats fed the experimental food to foods A, B, and C for Pyr. The cats fed the experimental food had significantly lower ICTP when compared with food B ($P < 0.01$). Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and total n-3 fatty acids were significantly higher for the cats fed the experimental food when compared with all 3 foods ($P < 0.01$).

Arthritic marker and serum fatty acid data for Day 90 are presented in Table 4. The cats fed the experimental food had significantly lower NTX when compared with the food B ($P < 0.01$) and food C ($P = 0.06$). No differences were observed when comparing the cats fed the experimental food to foods A, B, and C for Pyr. Cats fed the experimental food had significantly lower ICTP when compared with food B ($P = 0.02$). Eicosapentaenoic acid, DHA and total n-3 fatty acids were significantly higher for the cats fed the experimental food when compared with all 3 foods ($P < 0.01$).

Tekscan® pressure mapping are shown in Tables 5 and 6. Cats fed food B had a higher PVF when compared with cats fed the experimental food ($P = 0.04$) at Day 30. By Day 90, no differences were seen in PVF among all treatments.

Body weights and bone mineral content for Days 0 and 90 are presented in Table 7. Cats fed all the foods maintained body

Table 5. Tekscan Pressure Mapping in Cats on Day 45 Fed 4 Different Foods in Study 2.

Foot Measured	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	Vs A*	Vs B*	Vs C*
Right front, PVF	6.69	6.86	6.75	6.64	0.64	0.89	0.89
Left front, PVF	6.44	6.63	7.34	6.47	0.55	0.04	0.92
Right rear, PVF	5.41	5.73	4.90	5.16	0.29	0.17	0.40
Left rear, PVF	5.30	5.70	5.08	4.78	0.25	0.59	0.13

PVF = peak vertical force (newtons expressed as a percentage of body weight).
*Probability of greater F value.

Table 6. Tekscan Pressure Mapping in Cats on Day 90 Fed 4 Different Foods in Study 2.

Foot Measured	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	Vs A*	Vs B*	Vs C*
Right front, PVF	10.8	12.3	11.0	11.5	0.12	0.79	0.51
Left front, PVF	10.8	12.4	11.1	11.1	0.13	0.76	0.79
Right rear, PVF	8.2	9.2	7.6	8.3	0.28	0.49	0.96
Left rear, PVF	7.3	8.9	7.9	8.0	0.08	0.51	0.46

PVF = peak vertical force (newtons expressed as a percentage of body weight).
*Probability of greater F value.

Table 7. Body Composition Measured in Cats Fed 4 Different Foods in Study 2.

Body Parameter Measured	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	Vs A*	Vs B*	Vs C*
Weight Day 0, kg	4.44	4.84	4.64	4.77	0.30	0.60	0.39
Weight Day 90, kg	4.32	5.01	4.42	4.75	0.10	0.81	0.31
Weight change, kg	-0.12	0.17	-0.07	-0.02	0.13	0.78	0.59
Day 0 vs day 90*	0.36	0.20	0.63	0.88	–	–	–
Bone mineral content Day 0, g	118.1	131.2	123.7	131.7	0.33	0.67	0.30
Bone mineral content Day 90, g	116.5	129.1	122.6	127.1	0.32	0.99	0.40
Bone mineral content change, g	-1.35	-1.89	-1.07	-4.51	0.85	0.92	0.26
Day 0 vs Day 90*	0.53	0.30	0.57	0.02	–	–	–

*Probability of greater F value.

weight and bone mineral content throughout the study with the exception of food C. Cats fed food C had a significant decrease in bone mineral at Day 90 when compared with Day 0 (-4.51 g; $P = 0.02$).

DISCUSSION

The objective of these experiments was to identify potential arthritic markers for cats and to determine if wellness foods can be fed to geriatric cats to protect cartilage and/or prevent the onset of the disease. Previous studies investigating OA in dogs have reported similar gelatinase and collagenase activity in synovial fluid of healthy and arthritic dogs with those reported in humans.[1,8,16] These data suggest that there are parallels in the development of OA between species. As a result, the markers identified and utilized in Study 1 were chosen because of their success in the identification of arthritis in humans, dogs, and other species.[8,16-19] For a marker to be successful, the marker must differentiate between arthritic and non-arthritic cats, have low variability, and be reproducible. In Study 1, osteocalcin, BAP, and ICTP appeared to have the best potential for studying arthritis in cats because of the low variability and the significant differences observed between the 2 groups of cats. The difference between arthritic and non-arthritic cats for ICTP in Study 1 is similar to those reported in other species.[18-20]

The increased level of osteocalcin and BAP in non-arthritic cats observed in Study 1 was unanticipated. Besides cartilage damage, OA is normally associated with bone remodeling as well. As a result, osteocalcin and BAP were measured in Study 1 to determine how arthritis may alter bone remodeling in vivo. Osteocalcin and BAP were significantly higher in the non arthritic cat in Study 1. This is contrary to what is seen in humans with arthritis. However, studies in dogs may suggest that osteocalcin may behave differently in dogs with arthritis. Lajeunesse et al[21] found no differences in serum levels of osteocalcin and BAP between healthy and experimentally induced osteoarthritic animals. Yamka et al[17] found higher levels of osteocalcin and BAP in non-arthritic versus arthritic dogs. These data suggest that bone remodeling and/or damage may not alter or behaves differently in arthritic animals when compared with humans.

The purpose of the second study was to determine what effect nutrition has in cartilage protection and metabolism in geriatric cats. In the initial screening for Study 2, cats were radiographed to ensure cats were healthy and had no signs of OA. A random group of 40 geriatric cats were selected for the screening of this study. These cats appeared to show no signs of visual lameness and were radiographed to determine if any potential signs of OA existed. The radiographs determined that 20% of the cats had signs of OA even though no visual signs of OA were apparent. These results are consistent with Hardie.[2] Hardie[2] also found 20% of the cats (n = 68) older than 12 years had signs of OA. The findings in the current study also confirm that feline OA can often go undiagnosed because no visual signs of OA were evident.

Nutrition has been shown to influence developmental and degenerative orthopedic diseases and the inflammatory process of arthritis (feeding of omega-6 vs omega-3 fatty acids).[4] Therefore, geriatric cats were fed wellness foods to see if the foods could prevent and/or reduce the onset of age-related arthritis. Three commercially available age-appropriate foods and an experimental food were fed to determine the potential benefit of feeding these foods on cartilage health. The experimental food contained increased levels of EPA, methionine, manganese, and no synthetic GAG when compared with the 3 commercial foods. These 3 nutrients were increased because of the known cartilage health benefits in other species.[17]

The addition of EPA to foods has been shown to suppress production of pro-inflammatory cytokines and cartilage degradative

enzymes.[4,10,22] The feeding of the experimental food resulted in increased levels of EPA in the serum of the cats when compared with other treatments. This was expected because the EPA level of the experimental food was greater than 3 times the level in the other foods. Because the source of the EPA was fish oil, this also resulted in increased levels of DHA and total omega-3 fatty acids in the blood as well when compared with all treatments. These data indicate that differences in serum EPA, DHA, and omega-3 fatty acids can be seen at Day 30. Also, higher levels of these fatty acids in the blood can result in the potential reduction of inflammatory cytokines associated with OA.

The levels of methionine in the experimental food were also increased to provide increased levels of free sulfate for cartilage maintenance to the cat. Cartilage is a tissue that, after development, expands in size by deposition of collagen type II and tissue-specific GAG, which require a source of organic sulfate for synthesis. The mechanism by which chondroitin sulfate and other sulfate containing chondroprotective compounds is believed to be the result of overcoming a dietary deficiency in sulfur. The GAG are metabolized by the body to release free sulfate that is excreted via urine and is inversely related to the amount of sulfur containing amino acids in the diet.[23] The free sulfate is then available for utilization by the cartilage. Suboptimal amounts of inorganic sulfate used for de novo synthesis of GAG may not be a significant problem in healthy cartilage but may be detrimental in cases of animals with OA.[23] Therefore, an increase in methionine and/or cysteine in the foods could provide a potential benefit when formulating foods for healthy geriatric cats that do not contain any synthetic GAG.

Manganese was also supplemented at an increased level because of its role in proteoglycan biosynthesis. It has been previously demonstrated in poultry that the manganese deficiency can reduce the quantity and quality of cartilage proteoglycans.[24] Manganese is a cofactor in the synthesis of GAG and its supplementation would benefit the synthesis of cartilage matrix and synovial fluid.[10,25]

In the current study, it appears that these 3 nutrients when fed together may be beneficial for cartilage protection in cats. When comparing the cartilage markers across all treatments, the experimental food consistently had lower values for each marker analyzed throughout the experiment. To determine if cats showed signs of lameness, cats were walked across the Tekscan® mat to determine PVF in cats fed the 4 treatments. This measure of PVF can be a good indicator and has been shown to differ in dogs with and without signs of lameness.[12,13] The cats fed food B had a reduction in the amount of force exerted on the left front limb at Day 45. However, change in peak force was not seen at Day 90. These data suggest that the use of PVF in cats may not be a good indicator for lameness because of the high variability. The results of this study indicate that the experimental food is protecting the cartilage of the geriatric cat without the incorporation of synthetic glucosamine or chondroitin to the food.

As mentioned previously, OA is also associated with changes in bone structure. Thus, it became relevant to measure bone mineral content via DXA. The DXA scans were used to determine the effects of these foods on bone mineral content and if any correlation existed with measured bone mineral content and the markers measured in the blood. Throughout the trial, there were no observable changes in the bone mineral content as measured by DXA with the exception of the cats fed food C, and no correlation between DXA or the markers existed. This may suggest that the blood markers are good indicators of early signs of arthritis before changes in bone may be observed.

Obesity is often associated with developmental OA. Therefore, nutritional management becomes relevant in the treatment and/or prevention of OA.[26] All of these foods were effective in maintaining the

weight of the geriatric animals throughout the duration of the study. Thus, any changes observed in arthritic markers would not be associated with obesity or weight gain in this study.

The results of the current study demonstrate that geriatric cats can be fed wellness foods containing increased levels of EPA, methionine, manganese, and no synthetic glucosamine or chondroitin to delay and/or prevent the onset the signs of arthritis in cats. Because of the high variability of arthritic blood markers, it is apparent that studies investigating early signs of arthritis and/or arthritis treatment in cats should measure multiple markers to ensure that the data is interpreted properly.

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