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

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ABSTRACT

The objectives of these studies were to evaluate arthritic markers and to determine the effects of feeding wellness-type foods on cartilage protection in geriatric animals. Study 1 utilized 26 dogs to determine biochemical differences between arthritic and non-arthritic dogs. Study 2 utilized 40 geriatric dogs (>10 years of age) in an experiment to evaluate 4 wellness foods (experimental food, food A, B, and C) for cartilage protection. All foods were fed to groups of 10 dogs (40 dogs total) for a period of 90 days. In Study 1, arthritic dogs had increased serum type 2 collagen synthesis (CII; $P = 0.03$) compared with non-arthritic dogs. Osteocalcin (OC) concentration was reduced in arthritic dogs compared to non-arthritic dogs ($P = 0.02$). No differences were observed for other markers measured in Study 1. In Study 2, at Day 30, the experimental food had significantly lower OC when compared with food C ($P = 0.04$). No differences were observed among treatments for CII, cartilage oligomeric matrix protein (COMP), or amino terminal linked telopeptide (NTX) at Day 30. At Day 90, the experimental food had significantly higher OC when compared with food B ($P = 0.02$) and food C ($P < 0.01$). No differences were observed among treatments for COMP or CII. The experimental food had significantly lower NTX when compared with food A ($P = 0.04$). These data indicate that wellness foods can be developed without the use of synthetic glucosamine or chondroitin to protect the cartilage in the dog. Multiple arthritic markers must be used because of high variability in individual markers alone.

INTRODUCTION

Osteoarthritis (OA) is a complex disease process of articular cartilage that is associated with a variable degree of synovitis from natural aging, trauma, and/or disease.[1] Quality of life is typically compromised due to joint damage, reducing the dog's ability to play and interact with the pet owner. The disease is characterized by a loss of balance between synthesis and degradation of the articular cartilage. This loss of balance results in cartilage erosion and damage that leads to visual signs of lameness.[2] Serum and urinary biomarkers have been studied as a means to access cartilage damage under experimental conditions and to assess joint problems in clinical scenarios.[3-6]

The understanding of the differences in biomarkers for collagen degradation and joint tissue metabolism in dogs 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 dogs.

Many effective nutritional technologies (ie, n-3 fatty acids and glucosamine/chondroitin [GAG]) have been developed to overcome OA conditions in adult dogs.[7-9] An alternative approach would be to change the propensity for arthritis by altering joint tissue metabolism prior to the onset of arthritic conditions.[10] In order to develop intervention schemes, the basic physiological differences between arthritic and non-arthritic dogs need to be established to determine the intervention points that can be manipulated through nutrition.

Thus, more information is needed to determine the effects of nutrition on the prevention of arthritis in dogs. The objectives of these studies were: 1) to determine which arthritic markers could be utilized to determine onset of arthritis and 2) to evaluate the effects of feeding a 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 dogs (13 with signs of naturally occurring lameness and 13 without signs of lameness) were identified for the study. The dogs were cared for in accordance with Institutional Animal Care and Use Committee protocols. Radiographs were taken of the joint to confirm positive visual signs for degenerative joint disease. Radiographs were taken for the shoulders, hips, elbows, knees tarsus, carpus, and digits. The radiographs were scored as 0, 1, 2, or 3 based on the scoring system used by Hardie et al.[11]

Blood was drawn and collected, and the serum harvested and stored at -20ºC in 1-mL aliquots. The serum was analyzed for the following biomarkers using commercially available kits: matrix metalloproteinase 13 (MMP-13; Matrix metalloproteinase 13 ELISA, R&D Systems Inc., Minneapolis, Mn; Cat#F13M00), amino-terminal cross-linked telopeptide (NTX; Amino-terminal cross-linked telopeptide ELISA, Wampole Laboratories, Princeton, NJ; Cat#9021), type II collagen synthesis (CII; Type II collagen synthesis ELISA, IBEX, Montreal, Quebec, Canada; Cat#60-1003), bone-specific alkaline phosphatase (BAP; Bone-specific alkaline phosphatase ELISA, Quidel, San Diego, Calif;Cat#8012), total D-pyridinoline (D-Pyr; Total D-pyridinoline ELISA, Quidel, San Diego, Calif; Cat#8030), pyridinoline (Pyr; Pyridinoline ELISA, Quidel, San Diego, Calif; Cat#8019), osteocalcin (OC; Osteocalcin ELISA, Nordic Bioscience Diagnostics, Herlev, Hovedgade; Cat#3OSC4000) and carboxy-terminal cross-linked telopeptide (ICTP; Carboxy-terminal cross-linked telopeptide ELISA, Orion Diagnostica, Denmark; Cat#OD-06099).

Study 2

Dogs and Treatments

The study utilized 10 healthy geriatric beagle dogs (mean age 12.0 ± 1.0 years) per treatment group (40 dogs total). The dogs were cared for in accordance with Institutional Animal Care and Use Committee protocols. The dogs 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. The 30-day pre-feeding assured that all animals were not consuming foods with elevated levels of n-3 fatty acids, methionine, or manganese.

During the 30-day pre-feeding period, all dogs were fed a dry control food formu-

lated in accordance with the Association of American Feed Control Officials (AAFCO)[12] nutrient guide for dogs and balanced to meet maintenance requirements. During the last week of the pre-feeding period dual-energy x-ray absorptiometry (DXA; DXA-QDR-4500, Hologic, Inc., Waltham, Mass) scans and Tekscan® (HR Walkway Platform Pressure Measurement System, Tekscan®, Inc. Boston, Mass) pressure map analysis were performed. The dogs were then blocked by age, gender, and body fat percentage and assigned to 4 different treatment groups. Each group of dogs 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[12] nutrient guide for dogs and balanced to meet maintenance requirements. The 3 commercial foods included Royal Canin Canine Mature Medium Breed (Food A; containing synthetic GAG), Purina Dog Chow Senior (Food B) and Eukanuba Senior Maintenance (Food C; containing synthetic GAG). The nutrient composition of each food is presented in Table 1. Additionally, dogs were offered enrichment toys, received routine grooming and had daily opportunities for socialization with other dogs and people.

Sampling

Blood samples were collected on Days 0, 30, and 90 during the test period. Serum was harvested and stored at -20ºC in 1-mL aliquots and later analyzed for fatty acids, arthritic markers, and bone markers. Dogs were scanned by DXA at 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 dogs 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 dogs walk freely or while on a leash across a thin mat placed on the floor. The thin mat contains a thin-film tactile pressure/force sensor (4 sensors/cm^2) which 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 can be used to compare changes in the forces exerted by each step and then correlated to joint health and arthritis.[13,14]

Analyses

The serum was analyzed for the following biomarkers using commercially available kits: OC Osteocalcin ELISA (Nordic Bioscience Diagnostics, Herlev, Hovedgade; Cat#3OSC4000), CII (Type II collagen synthesis ELISA, IBEX, Montreal, Quebec, Canada; Cat#60-1003), NTX (Amino-terminal cross-linked telopeptide ELISA, Wampole Laboratories, Princeton, NJ; Cat#9021), and cartilage oligomeric matrix protein (COMP; Cartilage oligomeric matrix protein ELISA, MD Biosciences, St. Paul, Mn; Cat#A-COMP.96). Serum was also analyzed for eicosapentaenoic acid (EPA), docoshexaenoic acid (DHA), linoleic acid, and total n-6 fatty acids and n-3 fatty acids. All serum fatty acids were analyzed using a modified method described by Rodriguez-Palmero et al.[15]

Statistics

Data were analyzed using General Linear Models procedure of SAS[16] to determine treatment means. The experimental unit was dog. 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 dogs were radiographed to confirm the presence of arthritis and utilized to

Table 1. Analyzed Nutrient Profiles of the 4 Foods Utilized in the Study 2.

Nutrients, 100% Dry Matter Basis	Pre-feeding Food*	Experimental Food†	Commercial Food A‡	Commercial Food B§	Commercial Food C§
Crude protein, %	21.53	20.10	27.65	27.76	29.39
Fat, %	17.00	16.45	13.52	11.08	13.59
Calcium, %	0.79	0.71	0.79	1.28	1.35
Phosphorus, %	0.64	0.61	0.68	0.93	1.14
EPA, %	0.01	0.32	0.10	<0.01	0.10
DHA, %	<0.01	0.22	0.09	<0.01	0.08
Linoleic acid, %	3.02	4.00	2.92	1.90	2.60
Total n-3 fatty acids, %	0.83	1.30	0.48	0.13	0.41
Total n-6 fatty acids, %	3.02	3.96	3.10	1.79	2.66
Methionine, %	0.40	1.00	0.49	0.51	0.66
Manganese, ppm	17	87	77	71	69
Atwater energy, kcal/kg	3972	4048	3980	3326	3992

*Pre-feeding food ingredient list: corn meal, poultry meal, animal fat, soy mill run, flaxseed, corn gluten meal, egg, pal enhancer, potassium chloride, calcium carbonate, choline chloride, iodized salt, vitamin premix, L-tryptophan, glucosamine, taurine, dicalcium phosphate, L-lysine, mineral premix, L-arginine, and chondroitin sulfate.
†Experimental food ingredient list: corn meal, poultry meal, soybean meal, animal fat, pal enhancer A, flaxseed, soybean oil, fish oil, beet pulp, corn gluten meal, DL-methionine, pal enhancer B, potassium chloride, dicalcium phosphate, calcium carbonate, L-carnitine, choline chloride, vitamin E, L-lysine, vitamin premix, iodized salt, taurine, L-tryptophan, L-threonine, mineral premix, preservative, manganese sulfate
‡Royal Canin Canine Mature Medium Breed.
§Purina Dog Chow Senior.
§Eukanuba Senior Maintenance.

determine which markers were to be used in Study 2. The differences in arthritic markers between arthritic and non-arthritic dogs are presented in Table 2. Osteocalcin and NTX were significantly higher in the non-arthritic dogs ($P = 0.02$ and $P = 0.04$, respectively). Type 2 cartilage synthesis was significantly higher for the arthritic dogs ($P = 0.03$). No differences were observed between the arthritic and non-arthritic dogs for BAP, ICTP, Pyr, D-Pyr, and MMP-13.

Study 2

All dogs remained healthy throughout the duration of the experiment. No differences were observed in average daily intakes of the dogs. Average daily intake for the experimental food group was 219 ± 16 g, food A group was 214 ± 15 g, food B group was 256 ± 16 g, and food C group was 222 ± 17 g. Serum fatty acids, arthritic markers, and bone markers measured in the blood at Day 30 are presented in Table 3. At Day 30,

Table 2. Arthritic and Bone Markers Measured in Blood in Study 1.

Metabolite	Non-Arthritic Dogs	Arthritic Dogs	Non-Arthritic vs Arthritic (*P*-value)
Osteocalcin, ng/mL	19.42	4.71	0.02
Bone specific alkaline phosphatase, ng/mL	12.23	48.05	0.20
Amino terminal linked telopeptide, nM BCE	11.61	7.16	0.04
Carboxy terminal linked telopeptide, ng/mL	5.41	4.50	0.19
Pyridinoline, nM/L	4.12	3.90	0.76
D-pyridinoline, nM/L	17.82	12.57	0.45
MMP-13, ng/mL	2.10	2.85	0.10
Type 2 cartilage synthesis, ng/mL	627.58	782.14	0.03

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

Metabolite	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	SEM	Vs A* (*P*-value)	Vs B* (*P*-value)	Vs C* (*P*-value)
Osteocalcin, ng/mL	1.65	2.02	1.47	3.71	0.66	0.65	0.85	0.04
Type 2 cartilage synthesis, µg/mL	683	723	580	713	50.6	0.61	0.11	0.67
Cartilage oligomeric matrix protein, U/L	0.92	0.96	1.01	1.03	0.09	0.77	0.47	0.37
Amino terminal crosslink telopeptide, nM BCE	14.3	13.2	16.9	14.3	1.3	0.56	0.16	0.99
EPA, mg/dL	9.80	3.96	1.53	3.68	0.5	<0.01	<0.01	<0.01
DHA, mg/dL	16.6	10.0	3.9	11.7	0.8	<0.01	<0.01	0.01
Linoleic acid, mg/dL	62.3	59.3	50.3	50.2	2.6	0.40	<0.01	<0.01
Total n-6 fatty acids, mg/dL	107	126	104	114	4	<0.01	0.56	0.27
Total n-3 fatty acids, mg/dL	28.5	15.2	6.4	17.0	1.1	<0.01	<0.01	<0.01

*Versus experimental food.

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

Metabolite	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	SEM	Vs A* (*P*-value)	Vs B* (*P*-value)	Vs C* (*P*-value)
Osteocalcin, ng/mL	5.21	4.34	3.60	2.82	0.45	0.14	0.02	<0.01
Type 2 cartilage synthesis, µg/mL	906	817	874	874	29.2	0.09	0.41	0.46
Cartilage oligomeric matrix protein, U/L	1.62	1.68	1.69	1.84	0.12	0.76	0.70	0.21
Amino terminal crosslink telopeptide, nM BCE	16.9	19.8	18.0	15.6	0.9	0.04	0.44	0.35
EPA, mg/dL	9.02	3.70	1.51	3.52	0.44	<0.01	<0.01	<0.01
DHA, mg/dL	17.4	12.1	3.9	14.5	0.8	<0.01	<0.01	0.01
Linoleic acid, mg/dL	62.7	60.3	55.8	53.0	3.2	0.60	0.14	0.05
Total n-6 fatty acids, mg/dL	108	134	112	124	5	<0.01	0.59	0.05
Total n-3 fatty acids, mg/dL	28.9	17.7	6.9	20.0	10.0	<0.01	<0.01	<0.01

*Versus experimental food.

the experimental food had significantly lower OC when compared with food C ($P = 0.04$). No differences were observed when comparing the experimental food group to food groups A, B, and C for CII, COMP, or NTX. Eicosapentaenoic acid, DHA, and total n-3 fatty acids were significantly higher for the experimental food when compared with the other 3 foods ($P < 0.01$). Linoleic acid was significantly higher for the experimental food when compared with food groups B and C ($P < 0.01$).

Serum fatty acids, arthritic markers, and bone markers measured in the blood at Day 90 are presented in Table 4. At Day 90, the experimental food had significantly higher OC when compared with food B ($P = 0.02$) and food C ($P < 0.01$). No differences were observed when comparing the experimental food group to food groups A, B, and C for COMP or CII. The experimental food group had significantly lower NTX when compared with food group A ($P = 0.04$). Eicosapentaenoic acid, DHA, and total n-3 fatty acids were significantly higher for the experimental food group when compared with the other 3 food groups ($P < 0.01$). Linoleic acid was significantly higher for the experimental food group when compared with food group C ($P = 0.05$).

Tekscan® pressure mapping are shown in Tables 5 and 6. No significant differences were observed on Day 45 among all foods. On Day 90, the experimental food tended to be higher than food group C ($P = 0.09$) for left front foot.

Body composition data for Day 0 and Day 90 are presented in Table 7. Dogs fed all the foods maintained body weight, lean and bone mineral content throughout the study. Dogs fed the experimental food, food B and food C did not differ in fat between Day 0 and 90 ($P > 0.40$); however, dogs fed food A had a significant increase in body fat at day 90 when compared Day 0 (642 g of fat; $P = 0.01$). Dogs fed food A trended towards having a loss in lean (~170 g; $P = 0.14$).

DISCUSSION

The objective of these experiments was to identify potential markers for studying arthritis in dogs and to determine if foods can be fed to geriatric dogs 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 in healthy and arthritic dogs with those reported in humans.[1,5,17] 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.[5,17-19] For a marker to be successful, the marker must differentiate between arthritic and non-arthritic dogs, have low variability, and be reproducible. In Study 1, OC, NTX, and CII appeared to have the best potential for studying arthritis in dogs because of the low variability and the significant differences observed between the 2 groups of dogs; however, the difference

Table 5. Tekscan Pressure Mapping in Dogs on Day 15 Fed 4 Different Foods in Study 2.

Foot Measured	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	SEM	Vs A* (*P*-value)	Vs B* (*P*-value)	Vs C* (*P*-value)
Right front, PVF	125.6	132.1	132.0	118.4	9.2	0.62	0.63	0.58
Left front, PVF	130.7	135.8	129.7	121.7	9.6	0.71	0.94	0.51
Right rear, PVF	82.4	78.4	89.7	80.7	7.5	0.71	0.49	0.87
Left rear, PVF	87.8	80.3	84.3	80.5	7.3	0.47	0.74	0.49

PVF = peak vertical force (newtons per kg body weight).
*Versus experimental food.

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

Foot Measured	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	SEM	Vs A* (*P*-value)	Vs B* (*P*-value)	Vs C* (*P*-value)
Right front, PVF	129.5	124.9	98.6	112.0	13.9	0.82	0.12	0.38
Left front, PVF	129.8	123.9	97.5	112.6	13.5	0.76	0.09	0.38
Right rear, PVF	83.7	78.6	66.3	77.3	8.9	0.70	0.17	0.62
Left rear, PVF	88.2	76.0	70.3	78.0	10.1	0.40	0.21	0.47

PVF = peak vertical force (newtons per kg body weight).
*Versus experimental food.

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

Body Parameter Measured	Experimental Food	Commercial Food A	Commercial Food B	Commercial Food C	SEM	Vs A*	Vs B*	Vs C*
Weight Day 0, kg	12.07	11.91	12.20	11.68	0.67	0.86	0.90	0.69
Weight Day 90, kg	12.63	12.55	12.29	11.98	0.72	0.94	0.74	0.53
Weight change, kg	0.56	0.64	0.09	0.30	0.20	0.75	0.11	0.36
Day 0 vs Day 90 1	<0.01	<0.01	0.66	0.16	–	–	–	–
Lean Day 0, g	7792	7842	7768	7691	475	0.94	0.97	0.88
Lean Day 90, g	7790	7672	7814	7544	488	0.87	0.97	0.72
Lean change, g	2	-170	46	-147	112	0.30	0.76	0.37
Day 0 vs Day 90*	0.98	0.14	0.69	0.20	–	–	–	–
Fat Day 0, g	3936	3647	3576	3942	394	0.61	0.52	0.99
Fat Day 90, g	4133	4289	3465	3872	403	0.78	0.25	0.65
Fat change, g	196	642	-111	-70	239	0.20	0.37	0.44
Day 0 vs Day 90*	1	0.42	0.01	0.64	0.77	–	–	–
Bone mineral content Day 0, g	429.2	422.0	430.1	433.2	23.8	0.83	0.98	0.91
Bone mineral content Day 90, g	428.4	429.2	427.7	437.8	24.3	0.98	0.98	0.79
Bone mineral content change, g	-0.8	7.2	-2.4	4.6	5.0	0.26	0.83	0.45
Day 0 vs Day 90*	1	0.87	0.16	0.64	0.36	–	–	–

between arthritic and non-arthritic dogs for NTX in Study 1 is contrary to what has been previously observed in women.[20] It is uncertain why this marker behaved in this manner, resulting in the need to investigate it further in Study 2.

The biomarker MMP-13 had a numerically higher value for the arthritic group; however, a significant difference was not observed. As a result, MMP-13 was not chosen as a marker for Study 2 because of the high variability between the 2 groups. The elevated levels of MMP-13 and CII observed in Study 1 are the result of the breakdown in cartilage. The cartilage matrix consists of 2 major components, type II collagen and the proteoglycan aggrecan. Collagen fibrils provide tensile strength to maintain tissue integrity. Aggrecan is interwoven with the collagen fibrils and contributes to cartilage matrix compressive stiffness. Damage to type II collagen and loss of aggrecan are fundamental features of damage to articular cartilage in OA. This damage has been linked to proteolytic enzymes secreted by chondrocytes and synoviocytes. The matrix metalloproteinase family (ie, MMP-13) is responsible for the primary cleavage of the triple helix of type II collagen. As a result, type II collagen is typically increased in osteoarthritic cartilage.[17] This supports the data observed in Study 1.

The increased level of osteocalcin in non-arthritic dogs observed in Study 1 was unanticipated. In addition to cartilage damage, OA is normally associated with bone remodeling as well. As a result, osteocalcin and bone specific alkaline phosphatase were measured in Study 1 to determine how arthritis may alter bone remodeling in vivo. Osteocalcin was significantly higher in the non arthritic dogs in Study 1. This is contrary to what is seen in humans with arthritis; however, studies in dogs suggest that osteocalcin may behave differently in dogs with arthritis. Lajeunesse et al[21] found no differences in serum levels of OC and BAP between healthy and experimentally induced osteoarthritic animals.

The purpose of the second study was to determine what effect nutrition has in cartilage protection and metabolism. Nutrition has been shown to influence developmental orthopedic diseases (in young or aging dogs)[22] and the inflammatory process of arthritis (feeding of omega-6 vs omega-3 fatty acids).[7] Therefore, the objective of Study 2 was to investigate the effects of feeding foods to geriatric animals. In this study, geriatric dogs were fed 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 their potential benefit for cartilage health. Two of the foods contained synthetic GAG (guaranteed by the manufacturer to contain >400 ppm combined). 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 their known cartilage health benefits.

The addition of EPA to foods has been shown to suppress production of pro-inflammatory cytokines and cartilage degradative enzymes.[4,7] The feeding of the experimental food resulted in increased levels of EPA in the serum of the dogs 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. The source of EPA was fish oil, which also resulted in increased levels of DHA and total omega-3 fatty acids in the blood when compared with all treatments. Consequently, the serum omega-6 fatty acids were also lower in the dogs fed the experimental food. These data indicate that differences in serum EPA, DHA, omega-3 fatty acids, and omega 6-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 dog. Cartilage is a tissue that, after development, expands in size by deposition of collagen type II and tissue-specific glycosaminoglycans requiring a source of organic sulfate for synthesis.[23] 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 glycosaminoglycans are metabolized by the body to release free sulfate, which 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 dogs 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] This is the result of inhibition of endochondral osteogenesis at the growth plates/epiphyseal cartilage resulting in a reduction in the synthesis of proteoglycans. For example, chondroitin sulfate synthesis is regulated by manganese at 2 sites: 1) the polymerase in forming the glycosaminoglycan chain and 2) galactotransferase, which is required for the linkage of the polysaccharide chain to the protein.[25]

In the current study, it appears that these 3 nutrients when fed together may be beneficial for cartilage protection in dogs. 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 dogs showed early signs of lameness, dogs were also walked across the Tekscan® mat to determine PVF. The measure of PVF is a good indicator for early lameness and has been shown to differ in dogs with and without signs of lameness.[13,14] The dogs fed food B tended to have a reduction in the amount of force exerted on the front limbs at Day 90. These data suggest that these dogs were beginning to show signs of arthritis as well. The results of this study indicate that the experimental food is protecting the cartilage of the geriatric dog without the incorporation of dietary synthetic glucosamine or chondroitin to the food.

As mentioned previously, OA is associated with changes in bone structure. Thus, it became relevant to measure bone density via DXA. The DXA scans were used to determine the effects of these foods on bone density and if any correlation existed with measured bone density and the markers measured in the blood. Throughout the trial, there were no observable changes in the bone density as measured by DXA 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 also often associated with developmental OA. Therefore, nutritional management becomes relevant in the treatment and/or prevention of OA.[26] The DXA data revealed that these foods were effective in maintaining the weight of the geriatric animals throughout the duration of the study. Therefore, any changes observed in arthritic markers would not be associated with obesity or weight gain in this study. Another difficulty facing geriatric animals is the maintenance of lean body mass. Age-associated decline in lean body mass results from a loss of skeletal muscle protein resulting from a decrease in muscle protein synthesis. Because lean body loss is an issue, many gerontologists have argued that an increase in dietary protein may be the appropriate strategy to counteract lean body

loss.[27] The results of this study suggest that an increase in dietary protein may not be the answer. The experimental food contained 20.1% crude protein and was able to maintain lean body mass in this treatment group. The dogs fed foods A and C contained greater than 27.5% crude protein and dogs lost 159 g of lean body mass on average when fed these 2 foods. Thus, protein quantity may not be a key driver to maintaining lean body mass.

CONCLUSION

The results of the current study demonstrate that geriatric dogs can be fed 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 dogs. Because of the high variability of arthritic blood markers, it is apparent that studies investigating early signs of arthritis and/or arthritis treatment in dogs should measure multiple markers to ensure that the data is interpreted properly. Also, geriatric dogs can maintain lean body mass and bone mineral when fed lower levels of dietary protein, calcium, and phosphorous.

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