Prospective study of dilated cardiomyopathy in dogs eating non-traditional or traditional diets and in dogs with subclinical cardiac abnormalities

Supplemental Information

Table S1. Supplemental information on diet pulse and diet pulse/potato scores.

Diet Pulse and Diet Pulse/Potato Score

The ingredients of the diets being fed varied and some diets contained a single pulse in the top 10 ingredients (e.g., chickpeas), whereas others had multiple pulses in the top 10 ingredients (e.g., green peas, pea protein, and lentils). Others had multiple pulses but none in the top 10 ingredients. The exact amount of pulses in each diet was not available from manufacturers and there is no diet analysis that is specific for pulses or potatoes. Therefore, we attempted to quantify the "dose" of pulses and potatoes each dog was receiving by using diet pulse and pulse/potato scores. The ingredients in the main diet being eaten by each dog at the time of enrollment were recorded in order of appearance in the ingredient list of the diet at the time of enrollment. If the first ingredient on the ingredient list was a pulse, it was given a score of 25, the second ingredient got a score of 24, and so on down to the 25th ingredient, which got a score of 1. Ingredients after the 25th ingredient on the ingredient list did not get a score. For each appearance of a pulse or pulse fraction (e.g., peas, pea protein, pea starch, red lentils, green lentils), the corresponding number for its location on the ingredient list was added together to calculate the total pulse score. Therefore, if a diet had peas in the second position on the ingredient list (24), pea protein in the fourth position (22), and lentils in the tenth position (16), the total diet pulse score would be 62. If a dog was eating a consistent mixture of more than 1 diet, the diet with the highest pulse score was used. The compound used to preserve the fat or oil in diet was not counted as an ingredient (e.g., chicken fat preserved with mixed tocopherols would be counted as a single ingredient). A separate diet pulse/potato score was calculated by the same method except that potatoes and sweet potatoes were included in the score in addition to pulses. Labels or label information were collected from most dogs at the time of diagnosis. If the label was not collected from an individual dog, an internet archive website (Wayback Machine, https://archive.org/web/) was used to identify the ingredient list from the diet on the date as close as possible to the time of diagnosis. The ingredients of some diets changed markedly between the beginning and end of the study so, in some cases, the same diet could have a different score for an individual dog based on the dog's time of enrollment.

Table S2. Supplemental information on baseline blood testing in all dogs and DNA analysis in Doberman Pinschers and Boxers.

Variable	Sample	Laboratory	Results/Reference Range
Complete blood count	EDTA whole blood	In-house Clinical Pathology laboratories	---
Biochemistry profile	Serum	In-house Clinical Pathology laboratories	---
N-terminal B-type natriuretic peptide	EDTA plasma	IDEXX Laboratories, North Grafton, MA	• Reference range: 0-900 pmol/L (0-735 pmol/L for Doberman Pinschers)
High-sensitivity cardiac troponin I	Serum	Texas A&M University Veterinary Gastrointestinal Laboratory, College Station, TX	• Reference range: $\leq$0.06 ng/mL (older dogs <0.12 ng/mL)
Plasma taurine	Heparinized plasma	University of California Davis Amino Acid Laboratory, Davis, CA	• Low: $\leq$40 nmol/mL • No known risk for taurine deficiency: 41-59 nmol/mL (categorized as "borderline" in our study) • Normal: 60-120 nmol/mL • High: >120 nmol/mL
Whole blood taurine	Heparinized whole blood	University of California Davis Amino Acid Laboratory, Davis, CA	• Low: $\leq$150 nmol/mL • No known risk for taurine deficiency: 151-199 nmol/mL (categorized as "borderline" in our study) • Normal: 200-350 nmol/mL • High: >350 nmol/mL
DNA testing • Doberman Pinschers: NCSU DCM1 and NCSU DCM2 • Boxers: ARVC1	Cheek swab or EDTA whole blood	North Carolina State University Veterinary Genetics Laboratory, Raleigh, NC	• Negative • Positive heterozygous • Positive homozygous

Table S3. Supplemental information on selected nutritional variables also analyzed in the blood of small sub-groups of 18 dogs with DCM eating non-traditional diets and 18 healthy dogs (median [range]).[a] The number below the results indicates the number of dogs in each group tested. Comparisons were made between groups using Mann-Whitney U tests.

Variable	Dogs with DCM	Normal dogs	*P*-value
Thiamine (nmol/L)[b]	137 (102-228) 3	193 (183-211) 3	.51
Riboflavin (µg/L)[c]	40 (33-46) 2	40 (27-50) 3	1.00
Pyridoxal phosphate (nmol/L)[d]	459.2 (353.4-674.4) 3	726.2 (621.6-905.6) 3	.13
Choline (µM)[e]	10.6 (8.1-13.1) 4	8.2 (7.1-14.2) 4	.39
Betaine (µM)[e]	135.3 (92.2-160.4) 4	140.4 (108.6-273.3) 4	.77
Vitamin E (µg/mL)[f]	26.04 (12.94-32.41) 4	28.21 (24.14-72.93) 4	.56
Selenium (ng/mL)[g]	554 (487-585) 4	483 (437-553) 4	.15
Carnitine (plasma)[h] Total (µmol/L) Free (µmol/L) Esters (µmol/L) Ester/Free Ratio	 25.2 (10.7-40.3) 20.1 (7.1-31.8) 4.7 (3.6-9.4) 0.3 (0.2-0.5) 8	 34.8 (23.8-35.4) 28.3 (21.2-30.5) 4.3 (2.6-7.1) 0.1 (0.1-0.3) 3	 .54 .31 .84 .07
Carnitine (urine)[h] Total (µmol/L) Free (µmol/L) Esters (µmol/L) Ester/Free Ratio	 27.7 (13.8-42.0) 12.1 (4.0-28.1) 9.6 (8.9-27.0) 1.4 (0.3-2.5) 4	 17.7 (12.8-75.0) 12.2 (5.7-62.3) 9.0 (5.5-12.8) 0.8 (0.2-1.6) 5	 .62 1.00 .46 .22

In addition, an iron panel was analyzed in 4 dogs with DCM and compared to the laboratory's reference ranges for dogs. Bolded values were outside the reference range.

Variable	**Hematocrit (%)**	**Iron (µg/dL)[i]**	**Total iron binding capacity (µg/dL)[i]**	**Ferritin (ng/mL)[j]**
Reference range	39-55	73-245	270-530	89-489
Dog 1	49	235	424	**586**
Dog 2	48	**54**	497	**629**
Dog 3	49	111	339	317
Dog 4	54	194	361	**602**

[a]Mean age (DCM, 6.5±2.8 yrs; controls, 7.1±2.7 yrs; *P*=.47), mean body weight (DCM, 33.4±11.8 kg; controls 27.8±12.9 kg, *P*=.19), percentage of female and male dogs (DCM, 10 female and 8 male; controls, 12 female and 6 male; *P*=.37), and breed (*P*=.51) were not significantly different between groups. The most common breeds represented included Pit Bull (DCM, n=2, controls, n=4), Doberman Pinscher (DCM, n=3, controls, n=2), Boxer (DCM, n=2, controls, n=1), and German Shepherd (DCM, n=2, controls, n=1).

[b]High-performance liquid chromatography (ARUP Laboratories, Salt Lake City, UT)

[c]Liquid chromatography-tandem mass spectrometry (Mayo Clinic Laboratories, Rochester, MN)

[d]Enzymatic radioimmunoassay (Nutrition Evaluation Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA)

[e]Liquid chromatography-mass spectrometry (University of North Carolina-Chapel Hill Nutrition Obesity Research Center Metabolomics and Exposome Core, Kannapolis, NC)

[f]Ultra-performance liquid chromatography (Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI)

[g]Inductively-coupled plasma mass spectrometry (Michigan State University Veterinary Diagnostic Laboratory, Lansing, MI)

[h]Tandem mass spectrometry (University of California San Diego Biochemical Genetics Laboratory, San Diego, CA)

[i]Spectrophotometry (Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS)

[j]Enzyme-linked immunosorbent assay (Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS)

Table S4. Supplemental information on medications and taurine supplement administered to dogs with DCM over the course of the 9-month prospective study.

Medication	**Total (n=60)**	**Non-traditional diet group (n=51)**	**Traditional diet group (n=9)**	***P*-value**
Pimobendan	58 (97%)	49 (96%)	9 (100%)	1.00
Furosemide	47 (78%)	40 (78%)	7 (78%)	1.00
Angiotensin converting enzyme inhibitor	41 (68%)	37 (73%)	4 (44%)	.13
Amiodarone	17 (28%)	12 (24%)	5 (56%)	.10
Diltiazem	12 (20%)	8 (16%)	4 (44%)	.07
Spironolactone	10 (17%)	10 (20%)	0 (0%)	.33
Sotalol	6 (10%)	6 (12%)	0 (0%)	.58
Torsemide	5 (8%)	4 (8%)	1 (11%)	.57
Mexiletine	4 (7%)	3 (6%)	1 (11%)	.49
Carvedilol	4 (7%)	3 (6%)	1 (11%)	.49
Clopidogrel	1 (2%)	1 (2%)	0 (0%)	1.00
Digoxin	1 (2%)	1 (2%)	0 (0%)	1.00
Taurine supplement* (of 58 dogs discharged)	51 (88%)	44 (90%)	7 (78%)	.59

*Taurine supplementation was initiated at the time of the dogs' baseline visit for dogs discharged from the hospital using 1 of 4 brands (NOW taurine capsules, NOW, Bloomingdale, IL; Solgar taurine capsules, Solgar, Leonia, NJ; Twinlab taurine capsules, Twinlab, Boca Raton, FL, or Pet-Ag taurine tablets, Pet-Ag, Inc, Hampshire, IL) at a dose based on body weight: Dogs <10 kg were administered 250 mg q 12 hrs, dogs 10-25 kg were administered 500 mg q 12 hrs, and dogs >25 kg were administered 1000 mg q 12 hrs.