Received: 11 July 2022 | Accepted: 23 November 2022

DOI: 10.1111/jvim.16606

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Comparison of echocardiographic measurements and cardiac biomarkers in healthy dogs eating nontraditional or traditional diets

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Funding information
American College of Veterinary Internal Medicine Foundation; Oregon State University

Abstract

Background: There has been a recent association between nontraditional diets and development of diet-associated dilated cardiomyopathy (DCM) in dogs.

Hypothesis/Objectives: To compare echocardiographic measurements and cardiac biomarkers between healthy dogs eating nontraditional vs traditional diets. We hypothesized that dogs eating nontraditional diets would have lower measures of systolic myocardial performance compared to dogs eating traditional diets.

Animals: Forty-six healthy dogs: 23 eating nontraditional diets and 23 eating traditional diets.

Methods: Prospective, cross-sectional study. Dogs were divided into groups based on diet ingredients. Dogs underwent 2-dimensional (2D), 3-dimensional (3D), and Doppler echocardiographic examinations and analysis of plasma N-terminal prohormone of B-type natriuretic peptide, serum cardiac troponin I, and whole blood and plasma taurine concentrations.

Results: Mean 2D ejection fraction (EF) was lower for dogs eating nontraditional diets (48.65 ± 7.42%) vs dogs eating traditional diets (56.65 ± 4.63%; $P < .001$; mean difference 8.0% [4.0%-12.0%] 95% confidence interval [CI]). Mean 3D EF was lower for dogs eating nontraditional diets (45.38 ± 7.35%) vs dogs eating traditional diets (57.58 ± 4.84%; $P < .001$; 12.0% [8.0%-16.0%] 95% CI). Mean 2D left ventricular end-systolic volumes, indexed to body weight, were significantly higher in dogs eating nontraditional diets (1.46 ± 0.08 mL/kg) vs dogs eating traditional diets (1.06 ± 0.08 mL/kg; $P = .002$; 0.4 mL/kg [0.18-0.62 mL/kg] 95% CI).

Conclusions and Clinical Importance: Healthy dogs eating nontraditional diets had lower indices of systolic function and larger left ventricular volumes compared to

Abbreviations: 2D, 2-dimensional; 3D, 3-dimensional; cTnI, cardiac troponin I; DCM, dilated cardiomyopathy; EF, ejection fraction; FAC, fractional area change; FS, fractional shortening; GLS, global longitudinal strain; LAAo, left atrium to aortic root ratio; LAV, left atrial volume; LVEDV, left ventricular end-diastolic volume; LVEDVi, left ventricular end-diastolic volume indexed to body weight; LVESV, left ventricular end-systolic volume; LVESVi, left ventricular end-systolic volume indexed to body weight; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; RAV, right atrial volume; RAVi, right atrial volume indexed to body weight; SI, sphericity index; TAPSE, tricuspid annular plane systolic excursion (M-mode); TV S′, tissue Doppler imaging-derived peak systolic myocardial velocity of the lateral tricuspid annulus.

dogs eating traditional diets. Screening of apparently healthy dogs eating nontraditional diets might allow for early detection of diet-associated DCM.

KEYWORDS
canine, cardiology, diet, dilated cardiomyopathy, systolic function

1 | INTRODUCTION

Dilated cardiomyopathy (DCM) is the most common acquired cardiac disease of large and giant breed dogs, and the second most common acquired cardiac disease in all dogs.[1,2] The disease is characterized by systolic dysfunction with attendant ventricular dilatation.[3] Systolic dysfunction can lead to congestive heart failure, arrhythmias, and sudden cardiac death. The most common causes of DCM in dogs include hereditary and dietary factors. Causal genetic mutations are documented for DCM in Dobermans, Irish Wolfhounds, and Great Danes, among others.[4-8] Secondary causes of a DCM phenotype include nutritional deficiencies or intoxications, severe systemic illness and infection, and toxicoses.[9-13]

There has been an increase in the number of DCM cases associated with diet over the last several years, especially in atypical dog breeds.[14] 64%-95% of dogs with DCM in 4 recent studies were eating nontraditional diets, suggesting a possible association between DCM and nontraditional foods.[15-19] Except in 1 study that enrolled Golden Retrievers with taurine deficiency and DCM, taurine deficiency has been uncommon in other studies of diet-associated DCM.[15,18,19] Commonly associated with many of these diets was the use of pulse ingredients (eg, peas, lentils, chickpeas) and, to a lesser degree, potatoes and sweet potatoes.[20] Although a definitive causative role for nontraditional diets in the development of DCM is unproven, that dogs' myocardial performance can improve with diet change supports the role of diet.[21] Additionally, some studies document longer survival times for dogs diagnosed with DCM and congestive heart failure when having previously been eating a grain-free or nontraditional diet.[18,19]

Studies in healthy dogs eating nontraditional/grain-free diets document more cardiac enlargement with lower left ventricular fractional shortening (FS)[22] as well as higher concentrations of cardiac troponin I (cTnI) in serum and more frequent arrhythmias without echocardiographic differences between diet groups.[23] These reports evaluated few dog breeds and included some dog breeds predisposed to DCM with limited echocardiographic evaluation of systolic performance. No published studies have evaluated more extensive myocardial systolic performance indices in apparently healthy dogs of a broad spectrum of breeds that are representative of a community population eating different diet types. Determining any differences in cardiac size or myocardial systolic performance in apparently healthy dogs could help to better understand possible subclinical effects of nontraditional diets.

Therefore, the objective of this study was to compare echocardiographic measurements of myocardial performance and cardiac biomarkers in apparently healthy dogs eating nontraditional or traditional diets. We hypothesized that dogs eating nontraditional diets would have reduced indices of systolic function, and increased cardiac biomarkers compared to dogs eating traditional diets.

2 | METHODS

Forty-six apparently healthy dogs at least 3 years of age were enrolled in a prospective, breed- and age-matched, cross-sectional study. The study protocol underwent institutional review and owners provided informed consent before enrollment. The number of dogs was determined based on an a priori power analysis using a 2-tailed hypothesis where a clinically relevant reduction in systolic function could be detected with a power of 0.8 and significance of 0.05. This analysis showed that a minimum of 21 dogs per group was necessary for adequate statistical power, but we aimed for 23 dogs per group to ensure adequate numbers of eligible dogs. Dogs were owned by members of the Oregon State University College of Veterinary Medicine community, and additional dogs were recruited for study enrollment via social media. Study enrollment was incentivized based on a free of charge echocardiogram and cardiac biomarkers. Potential study subjects were evaluated by a complete diet history before enrollment. Ingredients as provided by the manufacturer were recorded for each dog's main diet, that is, the diet that provided the largest number of calories. Additionally, any dietary supplements being provided were recorded. An incomplete diet history resulted in study exclusion. Dogs eating raw meat or vegetarian diets were excluded from study consideration. Animals receiving daily medications or taurine supplementation were excluded from study consideration.

Dogs were considered healthy based on medical history and complete physical examination. A history of clinically relevant systemic illness or other medical disorders that, in the opinion of the investigators, could potentially confound echocardiographic results, also resulted in study exclusion. Abnormal cardiac auscultation, including presence of a heart murmur or a clinically important arrhythmia, or the echocardiographic presence of congenital heart disease resulted in study exclusion. Dog breeds with known or suspected genetic predispositions to DCM (Doberman Pinschers, Irish Wolfhounds, Great Danes, Newfoundlands, Portuguese Water Dogs, and Boxers) or taurine deficiency (Cocker Spaniels, Newfoundlands, Golden Retrievers) were excluded from the study. All dogs had to have been eating the same diet for at least 1 year before inclusion in the study, and >90% of daily caloric intake had to be provided by 1 extruded (kibble) diet.

Dogs were divided into respective groups based on the main diet's ingredient list. For the purposes of the study, diets were classified as traditional when they were grain-containing diets that did not contain potatoes or pulse ingredients among the top 10 ingredients on the ingredient list. Nontraditional diets were defined as those that contained pulse ingredients as main ingredients. Dogs eating nontraditional diets were first enrolled, and then an age- and breed-matched counterpart eating a traditional diet was subsequently enrolled. If an exact breed match could not be identified (n = 3), then a dog of a similar/near-matching breed was enrolled.

Complete 2-dimensional (2D), 3-dimensional (3D), and Doppler echocardiography with simultaneous ECG recording was performed on each included study subject (iE33, Philips Medical Systems, Andover, Massachusetts). Two-dimensional imaging was obtained with an 8-3 MHz phased array or X5-1 MHz matrix transducer. Three-dimensional imaging was performed using the X5-1 MHz matrix transducer. All echocardiograms were performed by a resident in training under the direct supervision of a board-certified veterinary cardiologist. Images were stored digitally and analyzed offline by use of a workstation with commercial software (TomTec Imaging Systems GmbH, Munich, Germany). The principal investigator (EO) performing the echocardiographic measurements was blinded to the diet status and laboratory results to reduce bias. Dogs that had valvular thickening with associated insufficiency that was not audible on physical examination were permitted in the study. Dogs were manually restrained without the use of sedation for the acquisition of left and right-sided images. If the dog did not tolerate manual restraint without the use of sedation for the acquisition of research-quality images, the dog was excluded from the study cohort.

The echocardiographic value recorded for each measurement was comprised of the average of 3 usually consecutive cardiac cycles. The blood-tissue interface (inner edge-to-inner edge technique) was used for all cardiac chamber evaluation. Left ventricular volumes at end-diastole (LVEDV) and end-systole (LVESV) in 2D were obtained from the right parasternal long-axis 4-chamber view.[24] Left ventricular end-diastole was defined as the echocardiographic frame at the time of mitral valve closure. Left ventricular end-systole was defined as either the frame before mitral valve opening or the minimum chamber dimension. Left ventricular volume was estimated in 2D by tracing the endocardial surface of the left ventricle at end-diastole and at end-systole in the right parasternal long axis view optimized for the left ventricle and employing the monoplane Simpson's method of discs.[25] Left ventricular papillary muscles (if visualized) were included in volume quantitation. Two-dimensional left ventricular end-diastolic (LVEDVi) and end-systolic (LVESVi) volumes were then normalized to body size by dividing by body weight. Ejection fraction (EF) was calculated using the formula ([LVEDV − LVESV]/LVEDV) × 100.

Linear left ventricular dimensions were measured in 2D at end-diastole (LVIDd) and end-systole (LVIDs) from the right parasternal short-axis view at the level of the papillary muscles. Measurements were performed starting from the midpoint of the septal arc to the left ventricular free wall, bisecting the 2 papillary muscles. The LVIDd and LVIDs were then normalized to body weight.[24] FS was calculated as ([LVIDd − LVIDs]/LVIDd) × 100. A diagnosis of reduced systolic function was based on a FS <25% or EF <40%.[26,27] Sphericity index (SI) was evaluated to assess for altered left ventricular geometry.[15] The SI was determined by measurements performed from the right parasternal long-axis 4 chamber view. The left ventricular length was measured 3 times in diastole at the start of the QRS complex from the left ventricular apex to a line across the mitral valve annulus. The left ventricular length was defined as a mean of these 3 measurements. The mean LVIDd measured in 2D was used for the left ventricular width. SI was then calculated as SI = left ventricular length/LVIDd.[27]

Left atrial volume (LAV) was measured in 2D from the right parasternal long-axis 4 chamber view.[24,28] The LAV was measured at ventricular end-systole in the last frame before mitral valve opening. The endocardial border of the left atrium was manually outlined, excluding the pulmonary veins and left atrial appendage. The ventral border of the left atrium was identified by the hinge points of the mitral valve. Monoplane Simpson's method of discs was used to estimate LAV. LAV was then indexed to body size by dividing by body weight. The left atrium-to-aortic root ratio (LA:Ao) was measured from the right parasternal short axis view at the level of the aortic root using previously described technique.[29]

For calculation of left ventricular volumes in 3D, a data set was acquired from the right parasternal long axis 4-chamber view by use of 4 consecutive ECG-triggered subvolumes integrated into 1 pyramidal full volume. Full volumes were analyzed off-line by use of commercially available software (TomTec Imaging Systems GmbH, Munich, Germany). The software automatically detected and traced the endocardial border at end-diastole. Manual adjustments of the LV endocardial border were then performed to optimize the tracing of the endocardial surface as described above. The software then automatically detected the endocardial border throughout the cardiac cycle, and manual adjustments were made of the endocardial border detection in each frame for end-diastolic and end-systolic measurements. Values for 3D LVEDV and 3D LVESV were calculated automatically by the software. Three-dimensional LVEDVi and LVESVi were calculated by dividing volume values by body weight. The software was then used to perform 3D strain analysis of the left ventricle with images acquired from the right parasternal long-axis 4-chamber view optimized for the left ventricle.[30-33] The region of interest was automatically detected by the software and manually adjusted to incorporate the entire left ventricular endomyocardium. The region of interest was evaluated to ensure it was visually synchronized with the cardiac movement throughout the cardiac cycle. The left ventricle was segmented in a 16-segment model by the software, and 3D speckle tracking analysis calculated global and regional strain components. Global longitudinal strain (GLS) was recorded.

Indices of right ventricular function were assessed from the left apical 4-chamber view optimized for the right ventricle. M-mode tricuspid annular plane systolic excursion (TAPSE), pulsed wave tissue Doppler imaging-derived systolic myocardial velocity of the lateral tricuspid annulus (TV S′), and right ventricular fractional area change (FAC) were measured as previously described.[34] Pulsed wave Doppler from the right parasternal short axis view at heart based was used to measure right ventricular outflow tract velocity. Pulsed wave Doppler from the left apical 5-chamber view was used to measure left

TABLE 1 Comparison of demographic variables of dogs eating nontraditional diets to dogs eating traditional diets

Variable	Nontraditional diet	Traditional diet	P value
n	23	23	-
Age (years)	6.38 ± 2.72	6.51 ± 2.24	.87
Sex			.02
Male	16 (16 castrated)	8 (7 castrated)	
Female	7 (6 spayed)	15 (12 spayed)	
Breed			1.00
Labrador retriever	7	5	
Mixed breed	5	5	
Border collie	3	3	
German shepherd	3	3	
Vizsla	2	2	
Australian shepherd	1	2	
Heeler	1	1	
Chesapeake Bay retriever	0	1	
Pointer	0	1	
Weimaraner	1	0	
Body weight (kg)	26.5 ± 9.7	23.9 ± 9.3	.35
Body condition score	5.1 ± .3	5.3 ± .5	.61

Note: Data are presented as mean ± SD. *P* values are for comparison between nontraditional and traditional diet groups.

ventricular outflow tract velocity. Measurements were determined by an average of 3 consecutive cardiac cycles.

Right atrial volume (RAV) was measured in 2D from the left cranial view optimized for the right atrium.[28] The RAV was measured at ventricular end-systole in the frame before tricuspid valve opening. The endocardial border of the right atrium was manually outlined, excluding the cavae and right atrial appendage. The ventral border of the right atrium was identified the hinge points of the tricuspid valve. Monoplane Simpson's method of discs was used to estimated RAV. RAV was then indexed to body size by dividing by body weight (RAVi).

Plasma and whole blood taurine concentrations were analyzed at a commercial laboratory (University of California Davis Amino Acid Laboratory, Davis, California) using blood collected in green-top lithium heparin tubes. Plasma and whole blood samples were stored at −80°C until batch analysis. Plasma N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) concentrations were analyzed through a reference laboratory using a commercially available assay (Cardiopet pro-BNP-Canine; IDEXX, Westbrook, Maine). Whole blood was collected in a lavender-top EDTA tube, centrifuged, and plasma was stored at −20°C until batch analysis. Serum cTnI concentrations were analyzed through a reference laboratory using a commercially available high-sensitivity assay (lower detection limit of 0.006 ng/mL; Advia Centaur CP Ultra-TnI; Texas A&M University Gastrointestinal Laboratory; College Station, Texas). Whole blood was collected in a red top tube, allowed to clot for 20 min, centrifuged, and serum was stored at −20°C until batch analysis.

Statistical analysis was performed with the use of commercially available software (SAS version 9.4, Cary, North Carolina). Sex distributions were compared between diet groups by a chi-square test while breed distributions were compared between diet groups by Fisher's exact test. Age and body weight were compared between diet groups by Student *t*-tests. All indices of cardiac functions and biomarkers were summarized descriptively. Histograms and Q-Q plots of residuals were examined to confirm the assumption of normality for each endpoint. Student *t*-tests were used to compare indices of cardiac functions and biomarkers between diets and sexes. Multiple comparisons were corrected for using the linear step-up false discovery method.[35] An adjusted multivariable *P* value < .05 was considered significant. Data are presented as mean ± SD.

3 | RESULTS

Twenty-three healthy dogs were enrolled in each diet group. Five additional dogs were screened before enrollment (3 in the nontraditional diet group, 2 in the traditional diet group) and excluded because of the presence of a heart murmur not detected before screening. One additional dog (traditional diet group) was excluded from the study because of noncompliance with gentle restraint for the echocardiographic study. There were no significant differences in age ($P = .87$), breed ($P = 1.00$), body weight ($P = .35$), or body condition score ($P = .61$) between the 2 diet groups (Table 1), however there were more male dogs in the nontraditional diet group than in the traditional diet group ($P = .02$).

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TABLE 2 Comparison of echocardiographic and laboratory variables of dogs eating nontraditional diets to dogs eating traditional diets

Variable	Nontraditional diet	Traditional diet	*P* value
Echocardiographic variables			
2D measurements			
LV EDVi (mL/kg)	2.83 ± 0.12	2.43 ± 0.11	.04
LV ESVi (mL/kg)	1.46 ± 0.08	1.06 ± 0.08	.002
LV ejection fraction (%)	48.65 ± 7.42	56.56 ± 4.63	<.001
LVIDdN	1.49 ± 0.04	1.43 ± 0.04	.37
LVIDsN	0.86 ± 0.03	0.76 ± 0.03	.07
LV fractional shortening (%)	43.28 ± 5.02	45.95 ± 5.74	.14
LV sphericity index	1.63 ± 0.04	1.78 ± 0.04	.01
LAVi (mL/kg)	1.18 ± 0.04	1.09 ± 0.06	.32
Left atrium to aortic root ratio	1.31 ± 0.03	1.30 ± 0.02	.87
3D measurements			
LV EDVi (mL/kg)	3.17 ± 0.11	2.55 ± 0.11	.001
LV ESVi (mL/kg)	1.72 ± 0.09	1.09 ± 0.08	<.001
LV ejection fraction (%)	45.38 ± 7.35	57.58 ± 4.84	<.001
LV global longitudinal strain (%)	−16.5 ± 0.8	−22.2 ± 0.8	<.001
Right ventricle fractional area change (%)	32.20 ± 8.85	39.40 ± 7.36	.02
TAPSE (mm)	14.0 ± 0.7	14.0 ± 0.7	.97
TV S′ (m/s)	0.12 ± 0.01	0.15 ± 0.01	.14
RAVi (mL/kg)	0.96 ± 0.04	0.82 ± 0.04	.13
Laboratory variables			
NT-proBNP (pMol/mL)	866 ± 90	736 ± 88	.38
cTnI (ng/mL)	0.072 ± 0.04	0.126 ± 0.21	.58
Whole blood taurine (nMol/mL)	271 ± 8	290 ± 12	.30
Plasma taurine (nMol/mL)	119 ± 8	116 ± 8	.87

Note: Data are presented as sex-adjusted mean ± SE. *P* values are for comparison between nontraditional and traditional groups.

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; cTnI, cardiac troponin I; EDVi, end-diastolic volume indexed to body weight; LAVi, left atrial volume indexed to body weight; LV, left ventricle; LVIDdN, left ventricular internal diameter at end-diastole normalized to body weight; LVIDsN, left ventricular internal diameter at end-systole normalized to body weight; NT-proBNP, N-terminal prohormone of B-type natriuretic peptide; RAVi, right atrial volume indexed to body weight; TAPSE, tricuspid annular plane systolic excursion (M-mode); TV S′, tissue Doppler imaging–derived peak systolic myocardial velocity of the lateral tricuspid annulus.

3.1 | Echocardiography

The mean 2D LVEDVi was significantly larger (difference in sex-adjusted means of 0.40 mL/kg with 95% confidence interval [CI] 0.07-0.73 mL/kg) in dogs eating nontraditional diets compared to those eating traditional diets ($P = .04$). The mean 2D LVESVi was significantly larger (0.40 mL/kg, 95% CI 0.18-0.62 mL/kg) in dogs eating nontraditional diets compared to those eating traditional diets ($P = .002$; Table 2). Two-dimensional EF was significantly lower (8.0%, 95% CI 4.0%-12.0%) in dogs eating nontraditional diets compared to those eating traditional diets ($P < .001$). Two dogs in the nontraditional diet group had a 2D EF below 40%, while no dogs in the traditional diet group had a reduced EF. No dogs in the study had a left ventricular FS less than 25%. The mean SI was significantly higher (0.15, 95% CI 0.05-0.25) in dogs eating nontraditional diets compared to dogs eating traditional diets ($P = .014$). No statistical differences were observed between groups for any of the other 2D echocardiographic measurements, including all measurements of left atrial size (LAV, indexed LAV, or LA:Ao).

The mean 3D LVEDVi was significantly larger (0.63 mL/kg, 95% CI 0.31-0.95 mL/kg) in dogs eating nontraditional diets compared to those eating traditional diets ($P < .001$). The mean 3D LVESVi was significantly larger (0.63 mL/kg, 95% CI 0.39-0.87 mL/kg) in dogs eating nontraditional diets compared to those eating traditional diets ($P < .001$). Mean 3D EF was significantly lower (12.0%, 95% CI 8.0%-16.0%) in dogs eating nontraditional diets compared to those eating traditional diets ($P < .001$). Mean GLS was significantly lower (5.68%, 95% CI 3.4%-8.0%) in dogs eating nontraditional diets compared to those eating traditional diets ($P < .001$). Six dogs in the nontraditional group had a reduced EF, while no dogs in the traditional diet group had a 3D

EF < 40%. The mean right ventricular FAC was significantly lower (7.0%, 95% CI 2.0%-13.0%) in dogs eating nontraditional diets compared to dogs eating traditional diets ($P = .02$). No statistical differences were observed between groups for TAPSE, TV S′, or RAVi.

3.2 | Laboratory analysis

No significant differences between the diet groups were observed in NT-proBNP, cTnI, or whole blood or plasma taurine concentrations. One dog in each diet group had a whole blood taurine concentration below established reference intervals, while no dog in the study had a plasma taurine concentration below reference intervals.

4 | DISCUSSION

In this study of apparently healthy dogs, those eating nontraditional diets had lower systolic function compared to those eating traditional diets using 2D and 3D echocardiographic measurements of EF, as well as GLS. Echocardiographic variables demonstrative of systolic function for dogs eating nontraditional diets were largely within extant reference intervals, but were nonetheless lower in dogs eating nontraditional diets.[3,24,25,27,34,36-40] A recent prospective study that evaluated dogs with subclinical myocardial dysfunction noted that dogs eating nontraditional diets had small but significant improvements in systolic function, left ventricular size, and left atrial size 9 months after a change to a traditional diet.[21] While these findings could support a role for diet in diet-associated DCM, longitudinal research is required to determine if these differences in systolic function in apparently healthy dogs eating nontraditional diets represents an early stage of DCM.

Although 2D measurements of linear left ventricular dimensions were not significantly different between diet groups, 2D and 3D left ventricular volumes at both end-diastole and end-systole were significantly larger in the nontraditional diet group. This suggests that volumetric measurements might be more sensitive to early changes in ventricular size associated with diet, although this requires further research in larger samples. Alternatively, this finding could reflect a true absence of differences between diet groups in the linear measurements.

Dogs eating nontraditional diets had lower left ventricular EFs as measured via 2D and 3D imaging in our study. We elected to calculate the left ventricular volume using the modified Simpson's method of discs based on American Society of Echocardiography guidelines as well as a study in Dobermans, which found the disc method was superior to linear quantification using M-mode for diagnosis of DCM.[25,41] We chose to index measured volumes to body weight based on a recent study noting volumetric measurements measured using the modified Simpson's method of discs related better to body weight than to body surface area.[40] While the left ventricular volumes and volumetric EFs obtained in our study were principally within established reference intervals, the significant differences observed between diet groups could reflect early impaired systolic left ventricular myocardial performance in dogs eating nontraditional diets. Of note, we did have dogs in both study groups with echocardiographic variables that fell outside of established referenced intervals. Potential explanations can include individual variability, day-day variability, utilization of non-breed specific reference intervals,[42] varying degrees of sympathetic stimulation, inclusion of athletic dogs, or preclinical primary or secondary DCM because of an alternative etiology.

In a recent study evaluating echocardiographic indices of myocardial function, biomarker concentration, and taurine status in 4 breeds of dogs eating nontraditional and traditional diets, no echocardiographic differences were found between groups using similar methods of echocardiographic assessment as this study (eg, modified Simpson's method of disks).[23] One important methodological difference is that the current study excluded dogs with a genetically based predisposition to the development of DCM. Other possible reasons for the differences in echocardiographic findings might include the differences in study sample (eg, inclusion criteria, number of subjects), duration of diet consumption before study enrollment, slightly different categorization of diets in each study, and specific diets consumed by the subjects of each study.

A recent study that evaluated Golden Retrievers eating nontraditional diets compared to those eating traditional diets identified differences in linear assessment of left ventricular size and function, in contrast to our findings.[22] In the current study, we evaluated multiple breeds with substantial variability between breed and body weight, whereas the single breed reduces variability, increasing likelihood of significant differences between diet groups in the study of Golden Retrievers. Though this previous study evaluated a different cohort of dogs utilizing different methodologies for measures of left ventricular size and function, the similar findings in our study of reduced myocardial systolic performance observed in dogs of multiple breeds eating nontraditional diets provides further corroborating evidence for this association.

In addition to lower indices of systolic function and larger measures of left ventricular volumes in our study, the nontraditional diet group also had a lower SI compared to those eating traditional diets. This is consistent with a previous study of dogs with DCM eating 1 specific grain-free diet compared to dogs with DCM eating grain-based diets,[15] although SI was not significantly different between diet groups in the 1 other study of dogs with diet-associated DCM that reported this variable.[21] SI was not reported in the 2 studies of apparently healthy dogs eating nontraditional/grain-free diets.[22,23] We found no differences in left (either volumetric or linear LA:Ao) or right atrial size (RAVi) between diet groups which is similar to the 2 previous studies in apparently healthy dogs.[22,23] We also noted that dogs eating nontraditional diets had a lower right ventricular FAC compared to dogs eating traditional diets. Other measures of right ventricular function (TAPSE and TV S′) were not different. To date, no published studies of diet-associated DCM (or apparently healthy dogs eating nontraditional diets) have measured right heart function. Further evaluation of right ventricular structure and function would be valuable in future studies of diet-related DCM.

Our study did not find any significant differences in cardiac biomarkers between diet groups. This is consistent with findings for NT-proBNP concentrations in a similar study of apparently healthy dogs eating nontraditional diets, but different from that study's findings of higher cTnI concentrations.[23] This could be because of the much smaller sample size in our study (n = 46) compared to the previous study that found higher cTnI concentrations (n = 188) and also to the wider variation in breeds in the current study. The lack of difference in NT-proBNP in both studies could be related to the absence of meaningful cardiac dilatation present in either study.

Neither whole blood nor plasma taurine concentrations were significantly different between diet groups in the current study. One dog in each diet group had a whole blood taurine concentration below the reference interval, and no dogs in either diet group had a plasma taurine concentration below the reference interval. This finding is in accord with recent studies of the possible link between nontraditional diets and diet-associated DCM in dogs.[15,18,19,23] Except in 1 study[22] of Golden Retrievers with DCM and taurine deficiency, few, if any, dogs with DCM in recent studies have had taurine deficiency. These findings suggest that systemic taurine deficiency is unlikely to play a major role in the development of diet-associated DCM, although it could have a secondary role and is still possible as a cause of DCM with specific commercial diets or in unbalanced home-prepared diets.

Our study has important limitations to address. One limitation is that this was a single, cross-sectional evaluation and not longitudinal in nature, thus the impact of individual and day-to-day variability must be considered, and we cannot report on possible disease progression or outcomes. The small study size limited the statistical power of the study which could have prevented identification of some possible differences in cardiac biomarkers and other echocardiographic measurements. Dogs were determined to be apparently healthy based on history and physical examination, but we did not perform blood pressure measurements, laboratory testing, electrocardiograms, or ambulatory electrocardiography as these tests were beyond the scope and budget of the study. Thus, conditions that could adversely affect the variables measured, such as myocarditis, hypothyroidism, systemic hypertension, or primary arrhythmic cardiac conditions, were not excluded before enrollment in the study. The findings in this study might not be generalizable to all dogs as we excluded dogs with cardiac murmurs and dog breeds predisposed to DCM to eliminate breed and pre-existing heart disease as confounding factors. This exclusion was intentional as we aimed to evaluate dogs in a true pre-clinical period, particularly given that DCM can be difficult to detect at these stages.[27,43,44] A further limitation is related to the unknown mechanism of recent diet-associated DCM in many dogs. Investigators in previous studies have used differing qualifiers and terminologies to

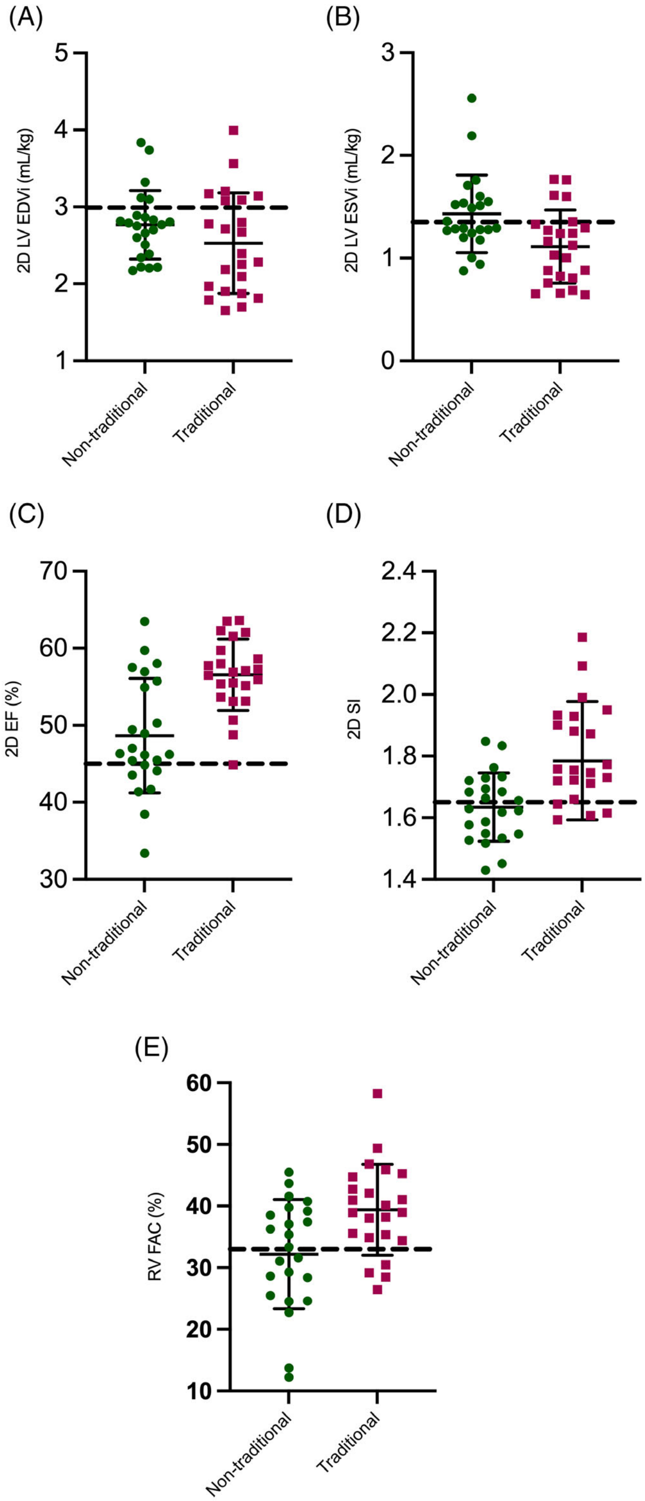

FIGURE 1 Comparison of two-dimensional (2D) echocardiographic variables between dogs eating nontraditional diets and dogs eating traditional diets. (A) 2D left ventricle end-diastolic volume indexed to body weight (2D LV EDVi). Dashed line at 2.99 mL/kg indicates upper limit of reference interval for 2D end-diastolic ventricular volume.[22] (B) 2D left ventricle end-systolic volume indexed to body weight (2D LV ESVi). Dashed line at 1.35 mL/kg indicates upper limit of reference interval for 2D end-systolic ventricular volume.[22] (C) 2D ejection fraction (2D EF). Dashed line at 40% indicates threshold for low EF.[25] (D) 2D sphericity index (2D SI). Dashed line at 1.65 indicates threshold for increased sphericity.[25] (E) 2D right ventricular fractional area change (RV FAC). Dashed line at 33% indicates threshold for reduced right ventricular fractional area change.[32]

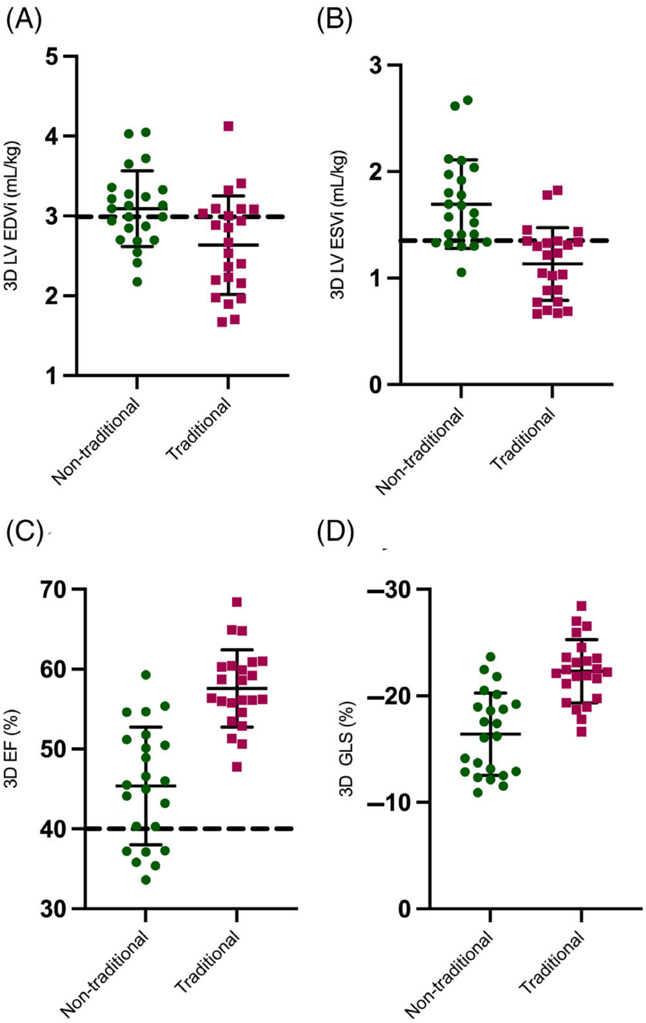

FIGURE 2 Comparison of 3-dimensional (3D) echocardiographic variables between dogs eating nontraditional diets and dogs eating traditional diets. (A) 3D left ventricle end-diastolic volume indexed to body weight (3D LV EDVi). Dashed line at 2.99 mL/kg indicates upper limit of reference interval for 2D end-diastolic ventricular volume (in healthy dogs).[22] (B) 3D left ventricle end-systolic volume indexed to body weight (3D LV ESVi). Dashed line at 1.35 mL/kg indicates upper limit of reference interval for 2D end-systolic ventricular volume (in healthy dogs).[22] (C) 3D ejection fraction (3D EF). Dashed line at 40% indicates threshold for low EF.[25] (D) 3D global longitudinal strain (3D GLS).

define nontraditional diets. To date, many of these studies (including ours) have utilized diet ingredients as a tool to distinguish 1 group of diets from another. This method unfortunately is definitionally inadequate until an underlying etiology can be elucidated. Finally, we did not adequately control for sex of participants. Therefore, while dogs were well-matched in terms of age and breed, the nontraditional diet group had significantly more male dogs than the traditional diet group. This could be relevant as primary DCM appears to have a male predisposition.[1,2] However, studies of diet-associated DCM have not had this male predisposition and have had nearly equal male: female numbers.[15,18,19,23]

Despite these limitations, results from this study showed that dogs eating nontraditional diets had several echocardiographic variables suggestive of larger left ventricular size and lower myocardial systolic performance as compared to dogs eating traditional diets, although echocardiographic variables for both groups were largely within extant reference intervals (Figures 1 and 2).

ACKNOWLEDGMENT

This study was supported by an ACVIM Cardiology Resident Research Grant, as well as a grant from the Department of Clinical Sciences at Oregon State University. Acknowledgment to Deborah Keys, Ph.D. for assistance in statistical analysis, Nadette Stang for laboratory sample collection, handling, and storage.

CONFLICT OF INTEREST DECLARATION

In the last 3 years, Dr. Freeman has received research funding from, given sponsored lectures for, or provided professional services to Aratana Therapeutics, Elanco, Guiding Stars LLC, Nestlé Purina PetCare, P&G Pet Care (now Mars), and Royal Canin. Dr. LeBlanc has given a sponsored lecture for Hill's Pet Nutrition. Authors declare no conflict of interest. The other authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Oregon State University IACUC approval #5158.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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How to cite this article: Owens EJ, LeBlanc NL, Freeman LM, Scollan KF. Comparison of echocardiographic measurements and cardiac biomarkers in healthy dogs eating nontraditional or traditional diets. *J Vet Intern Med*. 2023;37(1):37-46. doi:10.1111/jvim.16606