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# Effect of diet change in healthy dogs with subclinical cardiac biomarker or echocardiographic abnormalities

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## Abstract

**Background:** A recent study showed higher high-sensitivity cardiac troponin I (hs-cTnI) concentrations in healthy dogs eating grain-free (GF) compared to those eating grain-inclusive (GI) diets.

**Hypothesis/Objectives:** Healthy dogs with subclinical cardiac abnormalities eating GF diets at baseline will show improvements in cardiac biomarkers and echocardiographic variables after diet change, whereas healthy dogs eating GI diets at baseline will not improve.

**Animals:** Twenty healthy dogs with subclinical cardiac abnormalities (12 Golden Retrievers, 5 Doberman Pinschers, 3 Miniature Schnauzers).

**Methods:** This prospective study included dogs with increased hs-cTnI or N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations, or echocardiographic abnormalities. Mixed modeling was used to evaluate echocardiographic, hs-cTnI, and NT-proBNP differences between groups (GF or GI diet at baseline) over time (1 y after diet change).

**Results:** Ten GF and 10 GI dogs were evaluated. There were statistically significant time: group interactions for hs-cTnI ($P = .02$) and normalized left ventricular internal systolic diameter (LVIDsN; $P = .02$), with GF dogs showing larger decreases in these variables than GI dogs. Median (range) hs-cTnI (ng/mL) for GF dogs was 0.141 (0.012-0.224) at baseline and 0.092 (0.044-0.137) at 1 y, and for GI dogs was 0.051 (0.016-0.195) at baseline and 0.060 (0.022-0.280) at 1 y. Median LVIDsN for GF dogs was 1.01 (0.70-1.30) at baseline and 0.87 (0.79-1.24) at 1 y, and for GI dogs was 1.05 (0.84-1.21) at baseline and 1.10 (0.85-1.28) at 1 y.

**Conclusions and Clinical Importance:** Decreased hs-cTnI and LVIDsN in GF dogs after diet change supports reversibility of these subclinical myocardial abnormalities.

KEYWORDS
grain-free, hs-cTnI, NT-proBNP, nutritional dilated cardiomyopathy, troponin

**Abbreviations:** DCM, dilated cardiomyopathy; EDVI, end diastolic left ventricular volume index; EF, ejection fraction; ESVI, end systolic left ventricular volume index; FS, fractional shortening; GF, grain-free; GI, grain-inclusive; hs-cTnI, high-sensitivity cardiac troponin I; LV, left ventricular; LVIDdN, normalized left ventricular internal diameter in diastole; LVIDsN, normalized left ventricular internal diameter in systole; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VPC, ventricular premature complexes.

## 1 | INTRODUCTION

Dilated cardiomyopathy (DCM) in dogs is associated with shortened survival after the development of clinical signs of congestive heart failure.[1,2] A number of different etiologies including genetic, nutritional, toxic, and infectious can cause the DCM phenotype of ventricular enlargement and poor systolic function.[3-8] Recent concerns surrounding a possible association of DCM with grain-free (GF) diets or diets with peas, lentils, or potatoes as main ingredients have arisen because of reports to the Food and Drug Administration in combination with peer-reviewed clinical studies.[9-13] Taurine and carnitine deficiencies are known to cause nutritional DCM,[8,14,15] but neither of these deficiencies appear to be strongly associated with disease in most dogs with suspected GF or pulse-rich diet-associated cardiac abnormalities.[12,13,16-18] Although the underlying reasons for cardiac abnormalities in some dogs eating GF diets or diets with peas, lentils, or potatoes as main ingredients are not known, we recently reported higher high-sensitivity cardiac troponin I (hs-cTnI) concentrations in healthy dogs eating diets with those characteristics compared to dogs eating diets without those characteristics, which could be consistent with subclinical cardiomyocyte injury.[17]

Recent publications have reported that some dogs suspected to be affected by DCM associated with GF diets or diets with peas, lentils, or potatoes as main ingredients (even those dogs with congestive heart failure) can clinically and sometimes echocardiographically improve after transition to a grain-inclusive (GI) diet lacking pulses or potatoes as main ingredients.[10,12,13] Some of these dogs also have received taurine supplementation despite a lack of lower blood taurine concentration, however, and thus the role of taurine supplementation in clinical recovery of diet-associated DCM in this situation remains unknown. Although improvement in cardiac function over time is not expected with genetically-based DCM, the use of positive inotropic agents and lack of definitive test for diet-associated DCM have obscured interpretation of improved systolic function after diet change.[11-13]

The objective of our prospective study was to determine if diet change modulates cardiac biomarkers (hs-cTnI or N-terminal pro-B-type natriuretic peptide [NT-proBNP]), or echocardiographic variables over a 1-y period in apparently healthy dogs with subclinical abnormalities that were evaluated as part of a previous cross-sectional study.[17] We hypothesized that healthy dogs with cardiac biomarker or echocardiographic abnormalities that were eating GF diets at baseline would show improvements in these variables after diet change, but that dogs eating GI diets at baseline would not show improvements after diet change.

## 2 | METHODS

This prospective study was approved by the Institutional Animal Care and Use Committee at the University of Florida, College of Veterinary Medicine. (201810504). All dog owners provided informed consent. Enrollment was offered to owners of dogs that participated in a previous study[17] and met pre-defined inclusion criteria.

### 2.1 | Inclusion criteria

Owners of clinically healthy dogs evaluated in a previous study[17] were offered enrollment if their dogs had increased serum concentrations of hs-cTnI according to the laboratory-provided reference range (>0.06 ng/mL), increased NT-proBNP concentrations according to the laboratory-provided reference range (>900 pmol/L for Miniature Schnauzers and Golden Retrievers, and >735 pmol/L for Doberman Pinschers), or 1 of the following echocardiographic variables that were below or above predefined cutoff values: fractional shortening (FS) <25%, or normalized left ventricular internal dimension in diastole (LVIDdN) >1.8, or normalized left ventricular internal dimension in systole (LVIDsN) >1.2.[19] Dogs that met any of these criteria were permitted enrollment if the owners were willing to feed an intervention diet over a 1-y study period and agreed to return for scheduled re-evaluations. Enrollment was offered regardless of whether the dog was eating a GF or GI diet at initial evaluation, as long as the diet was not 1 of the intervention diets offered for transition.

Dogs with stable, treated hypothyroidism were eligible for study inclusion. Although taurine supplementation to address deficiencies in this amino acid was allowed, it was not recommended if blood taurine concentrations were within the normal reference range. Owners were allowed to continue the use of other dietary supplements.

### 2.2 | Procedures

#### 2.2.1 | Examination and diet history

Physical examinations were performed at baseline and every 3 mo after diet change. Body weight and body condition score (1-9 scale) were recorded at each visit.[20] Owners filled out a diet history form at each visit describing the type, amount, and duration of diet being fed as well as supplements or medications that were given.

#### 2.2.2 | Diets

The diets being fed at study enrollment were categorized as either GF or GI as previously described.[17] The owners of enrolled dogs were instructed to transition their dogs' diets slowly over 1-2 weeks to 1 of 6 intervention diets offered (Table S1A). Intervention diets for this study were GI, lacked pulses or potatoes in the top 25 ingredients and, for some, had been part of the recovery regimen for dogs with suspected diet-associated DCM in the authors' clinical practices. Six diets were offered to the owners of enrolled dogs to limit variability but still allow for owner preferences and specific dog needs, such as perceived allergies or overweight body condition. Only 1 diet per dog was allowed for the course of the study.

A pulse score was calculated for all diets in the study in an attempt to quantify pulses based on their position in the ingredient list and the number of pulses included. The top 25 ingredients were numbered in descending order from 25 to 1 and the assigned numbers

for all pulses or pulse fractions were summed to obtain the pulse score. For example, the top ingredient received a score of 25, the 2nd ingredient a score of 24, and so on until the 25th ingredient, which received a score of 1. A separate, pulse/potato score was calculated in the same manner to include pulses, pulse fractions, potatoes, sweet potatoes, or potato fractions.

#### 2.2.3 | Echocardiography

Standard echocardiography with concurrent ECG monitoring was performed without sedation at baseline and every 3 mo after diet change. Echocardiographic images and video loops were electronically stored for analysis after study completion. Left ventricular measurements were made in triplicate off-line after study completion by 1 of the investigators (DA) who was blinded to dog identity, visit date, and baseline diet type. Measurements included LVIDd and LVIDs obtained from right-parasternal short-axis M-mode, and left ventricular volume in diastole and systole generated using Simpson's monoplane method of discs from the right parasternal long-axis view.[21,22] Ejection fraction (EF) and FS were calculated, LVIDd and LVIDs were normalized to body weight using allometric scaling (LVIDdN and LVIDsN), and left ventricular volumes were indexed to body surface area to obtain EDVI (mL/m$^2$) and ESVI (mL/m$^2$) as previously described.[17,19,23] The rhythm during the echocardiogram was noted using the monitoring ECG. Twenty-four hour Holter monitoring was performed if clinically indicated, but was not part of the original study protocol.

#### 2.2.4 | Genotyping

Doberman Pinschers were tested for genetic mutations (DCM1 and DCM2) associated with DCM in this breed[4,5] as part of the previous study.[17]

#### 2.2.5 | Blood sampling

Blood was collected by peripheral venipuncture into 3 mL (Doberman Pinschers, Golden Retrievers) or 1 mL (Miniature Schnauzers) no additive tubes and ethylenediaminetetraacetic acid containing tubes for cardiac biomarker testing. Samples were verified visually as not hemolyzed before testing. Serum samples were immediately sent for analysis of hs-cTnI concentrations using the Gastrointestinal Laboratory, Texas A & M University (College Station, TX) using the ADVIA Centaur Troponin I Ultra analyzer (Siemens Healthcare Diagnostics, Deerfield, IL) an assay that has been validated for use in dogs.[24] Ethylenediaminetetraacetic acid plasma samples were immediately sent for analysis of NT-proBNP concentrations by IDEXX Laboratories (Westbrook, Maine). Lithium heparinized whole blood and plasma taurine concentrations were measured as part of the previous study[17] (corresponding to the baseline evaluation of the present study) at the Amino Acid Laboratory (University of California, Davis, Davis, CA) but were not serially monitored.

### 2.3 | Timeline for evaluations after diet change

After the dogs were fully transitioned to the intervention diet, the owners were instructed to bring their dogs back for reevaluation every 3 mo for a period of 1 y. Each of these visits included a physical examination, echocardiogram, and determination of hs-cTnI and NT-proBNP concentrations.

### 2.4 | Statistical analysis

Data are presented as means and SD if normally distributed and medians and ranges if nonnormally distributed. Differences between the GF and GI diet groups were examined using unpaired *t*-tests for normally distributed continuous data (or Mann-Whitney test for nonnormally distributed continuous data) and using Fisher's exact test or Chi-squared test for categorical data. The percentage of dogs examined at each intended time point and the actual time in months after diet change for each visit were calculated to quantify the impact of COVID-19 restrictions on data collection activities. Data from each visit after diet change (designated as V3, V6, V9, and V12 with the numbers denoting the intended months after diet change) were included in the final analysis if >50% of dogs were examined at the scheduled time point. Mixed modeling was used to evaluate differences in various categorical and continuous variables between baseline diet groups, changes over time, and interactions between baseline diet group and time (body weight, body condition score, hs-cTnI, NT-proBNP, LVIDdN, LVIDsN, FS, EDVI, ESVI, and EF). This analysis was performed controlling for breed, weight, and the intervention diet for all outcomes with the exception of weight, LVIDdN, and LVIDsN. Analyses of weight, LVIDdN, and LVIDsN were performed controlling only for breed and intervention diet because weight is already incorporated into these indices. Median values at each time point were graphed for those variables with statistically significant time: group interactions to visually illustrate directional changes over time for each group. Commercial software was used for all data analyses (SAS 9.4, Cary, North Carolina). $P < .05$ was considered statistically significant.

## 3 | RESULTS

### 3.1 | Study population

Figure 1 is a flow chart illustrating the number and breeds of dogs that were enrolled in the study between May 2019 and May 2021, and which dogs contributed to study attrition. Twenty-four dogs were enrolled (13 GF and 11 GI) but 3 dogs died before the first evaluation at 3 mo (2 GF, 1 GI), and 1 dog (GF) was excluded after enrollment

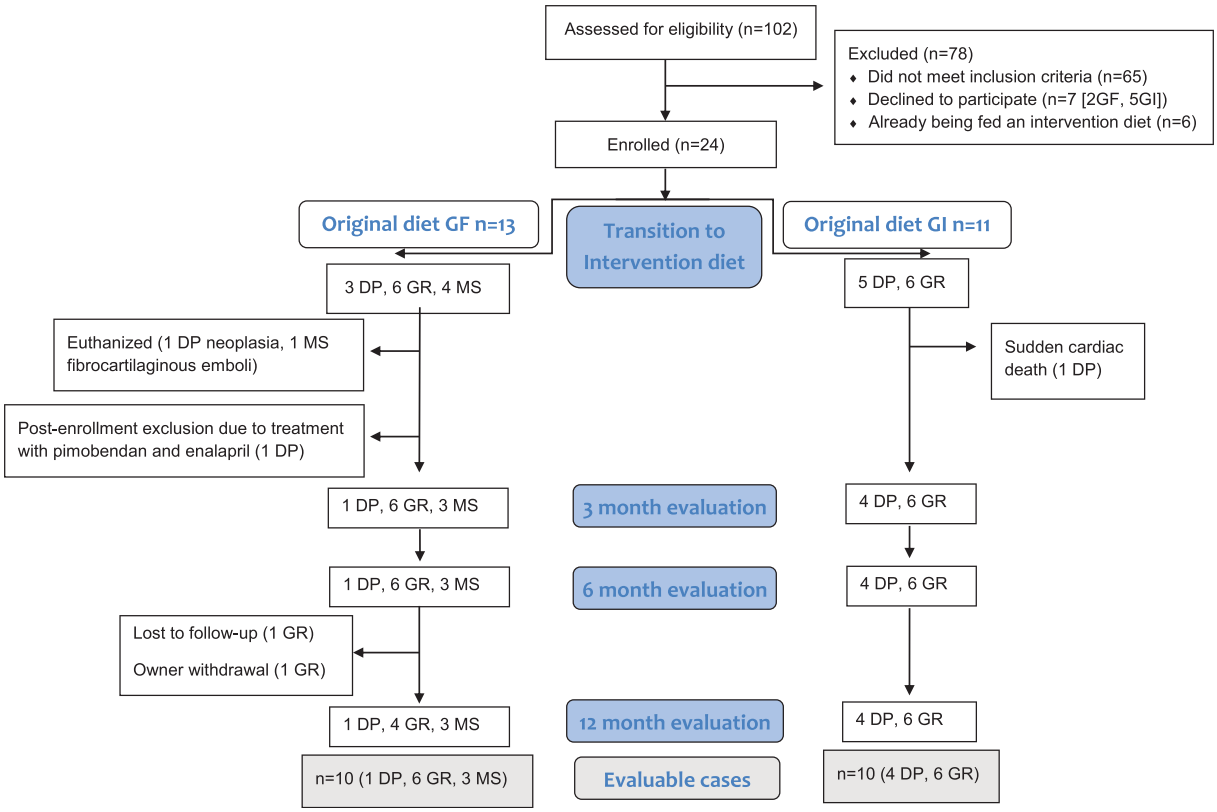


**FIGURE 1** The flow diagram illustrates the enrollment of dogs, reasons for exclusion, reasons for attrition, number of statistically evaluable cases, and the breeds for each of these categories

because pimobendan and enalapril were prescribed to treat systolic dysfunction. Two GF dogs completed the study to 6 mo, after which time 1 was lost to follow-up and the other withdrawn at owner request. Eight GF dogs and 10 GI dogs completed the study to 12 mo. The 2 GF dogs that completed the study to 6 mo were considered evaluable and were included in the mixed modeling analysis during statistical analysis.

No statistically significant differences were found in sex, age, breed, or reasons for enrollment at baseline between the evaluable GF and GI dogs (Table 1). Body weight at baseline was significantly lower for GF dogs compared with GI dogs (Table 1). Mixed model analysis of all study time points showed significant differences in body weight between the GF and GI groups ($P = .02$) but not over time or as an interaction after controlling for breed and intervention diet (Table 2). The weight difference between groups occurred because 3 Miniature Schnauzers (the only small breed studied) were part of the GF group and no Miniature Schnauzers were part of the GI group (Table 1). No statistically significant differences in body condition scores were present between groups, over time, or as an interaction term (Table 2).

## 3.2 | Visit timelines

Several study visits were not completed because of hospital caseload restrictions and owner-initiated rescheduling related to the COVID-19 pandemic. The percentage of dogs examined at each scheduled time after diet change was as follows: 3 mo (15/21 dogs; 71%), 6 mo (19/21 dogs; 90%), 9 mo (9/21 dogs; 43%), and 12 mo (18/21 dogs; 86%). Data from 0, 3, 6, and 12 mo were included in the statistical model because these time points included >50% of enrolled dogs. No differences were found between groups for the time of the visits (in months) after diet change (Table 1).

## 3.3 | Diets and supplements

Characteristics of diets fed to included GF and GI dogs at baseline are shown in Table S1B,C. The GF pulse score (median, 52; range, 0-80) was significantly higher than the GI pulse score (median, 0; range, 0-72; $P = .02$). The GF pulse/potato score (median, 96; range, 24-103) was higher than the GI pulse/potato score (median, 0; range, 0-78; $P = .006$).

One dog (GI Golden Retriever) received levothyroxine chronically for stable hypothyroidism and the dosage was not changed during the study.

Only 1 dog (GF Golden Retriever) had a whole blood taurine concentration below the reference range (192 nMol/mL; reference range, 200-350) but above the known deficiency concentration of 150 nMol/mL, and with a normal plasma taurine concentration (77 nMol/mL; reference range, 60-120 nMol/mL). All other dogs had both normal whole blood and plasma taurine concentrations. The owner of the dog with slightly low

**TABLE 1** Reason for enrollment, recheck times, and demographic data are shown for 10 grain-free (GF) and 10 grain-inclusive (GI) dogs (number of dogs or mean ± SD)

| | | GF | GI | *P* value |
|---|---|---|---|---|
| Reason for enrollment | hs-cTnI >0.06 ng/mL | 8 | 5 | .35 |
| | NT-proBNP >900 Golden Retriever and Miniature Schnauzer, >735 Doberman Pinscher | 1 | 5 | .14 |
| | FS <25% | 3 | 6 | .37 |
| | LVIDdN >1.8 | 1 | 0 | .99 |
| | LVIDsN >1.2 | 1 | 3 | .58 |
| Time (months) of rechecks after diet change | 3-mo visit | 3.2 ± 0.8 | 3.7 ± 0.6 | .16 |
| | 6-mo visit | 6.6 ± 0.9 | 6.7 ± 0.9 | .81 |
| | 12-mo visit | 12.3 ± 0.7 | 13.1 ± 1.5 | .24 |
| Sex | Female (intact or spayed) | 5 | 5 | 1.0 (overall) |
| | Male (intact or neutered) | 5 | 5 | |
| Breed | Golden Retriever | 6 | 6 | .09 (overall) |
| | Doberman Pinscher | 1 | 4 | |
| | Miniature Schnauzer | 3 | 0 | |
| Age (months) | | 67.5 ± 39.0 | 61.4 ± 32.6 | .60 |
| Weight (kg) | | 23.5 ± 10.3 | 34.6 ± 4.1 | **.01** |

*Note*: *P* values for statistically significant findings are bolded.
Abbreviations: FS, fractional shortening; GF, grain-free; GI, grain-inclusive; hs-cTnI, high-sensitivity cardiac troponin I; LVIDdN, normalized left ventricular internal diameter in diastole; LVIDsN, normalized left ventricular internal diameter in systole; NT-proBNP, N-terminal pro B-type natriuretic peptide.

whole blood taurine concentration gave taurine supplementation. Other dietary supplements given to the dogs are listed in Table S2.

### 3.4 | Cardiac biomarker findings

A statistically significant time: group interaction was found for serum hs-cTnI concentrations after controlling for the dogs' weight, breed, and intervention diet ($P = .02$, Table 2). The nature of this interaction is shown in Figure 2, which depicts median concentrations for each group at each time point. The median hs-cTnI concentration for the GF dogs decreased over time whereas the median hs-cTnI concentration for the GI dogs was stable to minimally increased over time, with convergence of the groups at the 6-mo evaluation.

A statistically significant group difference was found for NT-proBNP ($P = .04$), but no statistically significant differences were found over time, or as a time: group interaction, after controlling for the dogs' weight, breed, and intervention diet (Table 2).

### 3.5 | Echocardiography findings

Statistically significant time: group interactions were found for both LVIDsN ($P = .02$) and FS ($P = .01$; Table 2). The nature of these interactions is shown in Figure 3A,B, which depict median results for each group at each time point. The GF dogs showed a consistent decrease in median LVIDsN over the study period whereas results for the GI dogs fluctuated without apparent change during the study period. Median FS for the GF dogs increased over time more than the FS for the GI dogs, but temporal fluctuation in measurements occurred for both groups, resulting in 2 crossover points. The EDVI was significantly different between groups and changed significantly over time, but no time: group interaction was found (Table 2). No significant differences were found between groups, over time, or as time: group interactions for LVIDdN, ESVI, or EF, after controlling for the dogs' weight, breed, and intervention diet (Table 2).

### 3.6 | Electrocardiography findings

One GF dog (Golden Retriever) had single ventricular premature complexes (VPCs) noted on the monitoring ECG during echocardiography. These VPCs were frequent at V0, intermittent at V3, and not observed at subsequent visits. A 24-hour Holter monitor at V12 showed 21 single VPCs and 1 ventricular couplet, but a baseline Holter study was not available for comparison. Arrhythmias were not noted in any GI dogs.

### 3.7 | Genotyping

The GF Doberman Pinscher was DCM1 negative/DCM2 negative. The 4 GI Doberman Pinschers were DCM1 heterozygous positive/DCM2 negative ($n = 2$), DCM1 negative/DCM2 positive heterozygous ($n = 1$) and DCM1 homozygous positive/DCM2 heterozygous positive ($n = 1$).

**TABLE 2** Body weight and body condition score, cardiac biomarker concentrations, and echocardiographic variables over time are shown for both diet grain-free (GF) and grain-inclusive (GI) dogs

| | Baseline visit | | 3-mo visit | | 6-mo visit | | 12-mo visit | | Adjusted *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | GF ($n = 10$) | GI ($n = 10$) | GF ($n = 7$) | GI ($n = 5$) | GF ($n = 9$) | GI ($n = 8$) | GF ($n = 8$) | GI ($n = 10$) | Between groups | Over time | Time : group interaction |
| Body weight (kg) | 27.4 (6.8-35.4) | 35.0 (26.0-39.8) | 27.0 (7.1-35.8) | 35.0 (32.1-36.4) | 27.0 (7.8-35.0) | 34.9 (31.7-43.7) | 25.7 (6.8-37.4) | 34.9 (33.4-44.3) | **.02** | **.02** | .73 |
| Body condition score | 5.5 (4.0-7.5) | 5.0 (5.0-9.0) | 6.0 (5.0-7.0) | 5.5 (5.0-6.0) | 6.0 (5.0–7.0) | 6.0 (5.0-6.0) | 6.0 (5.0-8.0) | 5.0 (5.0-7.0) | .69 | .94 | .38 |
| hs-cTnI (ng/mL) | 0.141 (0.012–0.224) | 0.051 (0.016-0.195) | 0.126 (0.059-0.174) | 0.047 (0.020-0.199) | 0.068 (0.032-0.178) | 0.08 (0.016-0.297) | 0.092 (0.044-0.137) | 0.060 (0.022-0.280) | .26 | .30 | **.02** |
| NT-proBNP (pmol/L) | 446 (250-994) | 836 (250-1584) | 494 (250-1122) | 1046 (398-1643) | 632 (250-1403) | 914 (250-1729) | 507 (250-823) | 1038 (320-1754) | **.04** | .24 | .28 |
| LVIDdN | 1.58 (1.35-1.81) | 1.51 (1.35-1.72) | 1.57 (1.32-1.74) | 1.61 (1.34-1.69) | 1.48 (1.30-1.82) | 1.49 (1.28-1.69) | 1.50 (1.29-1.68) | 1.57 (1.33-1.78) | .08 | .40 | .27 |
| LVIDsN | 1.01 (0.70-1.30) | 1.05 (0.84-1.21) | 0.95 (0.68-1.33) | 1.12 (0.96-1.20) | 0.98 (0.70-1.28) | 0.96 (0.81-1.16) | 0.87 (0.79–1.24) | 1.10 (0.85-1.28) | .38 | .25 | **.02** |
| FS (%) | 30.7 (23.1-50.4) | 23.1 (18.8-34.3) | 35.4 (17.9-48.6) | 22.8 (18.5-31.8) | 29.1 (16.6-47.3) | 31.7 (19.3-35.9) | 35.9 (20.6-45.5) | 29.0 (17.5-35.3) | .83 | .75 | **.01** |
| EDVI (mL/m$^2$) | 60.7 (35.3-82.3) | 65.5 (50.9-84.5) | 52.6 (37.2-66.4) | 59.6 (52.0-65.3) | 61.1 (32.7-74.1) | 59.3 (38.3-67.6) | 49.4 (34.1-71.4) | 62.7 (49.3-80.9) | **.01** | **.02** | .43 |
| ESVI (mL/m$^2$) | 19.4 (13.1-44.0) | 29.8 (19.0-49.9) | 20.1 (13.2-35.8) | 25.3 (19.7-38.0) | 24.9 (9.4-38.0) | 27.0 (12.7-32.8) | 17.0 (8.2-38.9) | 29.7 (16.1-39.7) | .09 | .10 | .24 |
| EF (%) | 60.9 (44.8-72.5) | 53.3 (38.4-68.2) | 61.3 (42.7-64.4) | 57.6 (44.9, 61.7) | 59.3 (47.8-71.4) | 56.7 (43.6-66.7) | 61.9 (44.1-76.1) | 55.1 (40.4-67.4) | .69 | .89 | .12 |

*Note*: Data are presented as median (interquartile range). The adjusted *P* values were obtained from analyses which controlled for breed, weight, and intervention diet for all variables except body weight, LVIDdN, and LVIDsN, for which only breed and intervention diet were controlled for in the analysis. *P* values for statistically significant findings are bolded.

Abbreviations: EF, ejection fraction; FS, fractional shortening; GF, grain-free; GI, grain-inclusive; hs-cTnI, high-sensitivity cardiac troponin I; LVIDdN, normalized LV internal diameter in diastole; LVIDsN, normalized internal diameter in systole; NT-proBNP, N-terminal pro B-type natriuretic peptide.

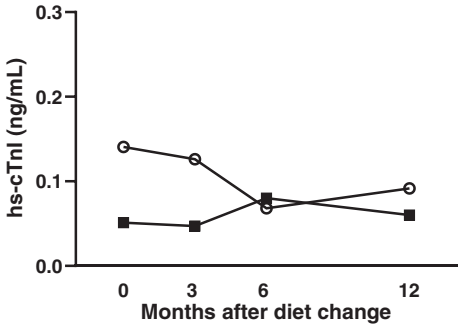


**FIGURE 2** Median high sensitivity cardiac troponin I (hs-cTnI) concentrations are shown at each time point after diet change for both diet groups. (○) Grain-free, (■) grain-inclusive. $P = .26$ between groups, $P = .30$ over time, $P = .02$ for time: group interaction

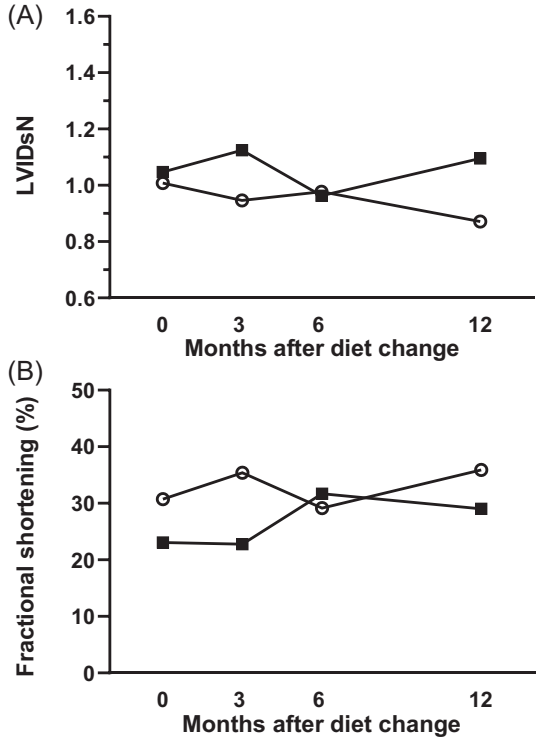


**FIGURE 3** (A) Median normalized left ventricular internal diameter in systole (LVIDsN) results are shown at each time point after diet change for both diet groups. (○) Grain-free, (■) grain-inclusive. $P = .38$ between groups, $P = .25$ over time, $P = .02$ for time: group interaction. (B) Median fractional shortening (%) results are shown at each time point after diet change for both diet groups. (○) Grain-free, (■) grain-inclusive. $P = .83$ between groups, $P = .75$ over time, $P = .01$ for time: group interaction

## 4 | DISCUSSION

Our main finding was that serum hs-cTnI concentrations and LVIDsN decreased over a 1-y period in dogs with subclinical cardiac abnormalities that were being fed GF diets at the time of enrollment and were transitioned to an intervention diet. The significant interaction between diet group and time for both of these variables was characterized by decreases in the GF dogs during the study period that did not occur in the GI dogs. Decreased serum hs-cTnI concentrations after diet change in GF dogs suggests decreased cardiomyocyte injury, whereas decreased LVIDsN suggests improved systolic performance, both of which indicate positive modulation by diet of cardiac structure and function in these dogs. The apparent improvement in ventricular arrhythmias in 1 GF dog after diet change is noteworthy, but it is difficult to draw conclusions without more affected dogs and a more complete and systematic cardiac arrhythmia evaluation.

The decreases in serum hs-cTnI concentrations and LVIDsN during the study period were observed in GF dogs but not in GI dogs, which supports the characterization of diets of concern that are based on GF designation. Importantly, however, there are other characteristics associated with GF status, including the use of pulses or potatoes that typically are used to replace grains. Although pulses and potatoes are also part of many GI diets and have been used in dog food formulations for many years,[18] the pulse score and pulse/potato score (which were used in an attempt to quantify the amount of pulses and potatoes) were both significantly higher in the GF diets compared to the GI diets. These ingredients or the amounts used could be linked to the changes in serum troponin concentrations we observed. Other, as of yet unidentified, factors associated with GF diets also could be important.

This finding expands results of the previous cross-sectional study which found higher hs-cTnI concentrations in dogs fed GF diets compared to dogs fed GI diets.[17] Although serum hs-cTnI concentrations from the GF dogs in that study were not markedly increased, other research in dogs and people indicates that even low-level, subclinical increases in hs-cTnI concentration can indicate cardiomyocyte injury secondary to noncardiac disease, diet, and cardiotoxic drugs, and can adversely impact prognosis.[25-29] The decrease in hs-cTnI concentrations after diet change in GF dogs in our study suggests that these low-level increases at baseline were not necessarily normal for these dogs and might be clinically important.

The ability of diet to modify disease is not a new concept. People who are at risk for cardiovascular disease can lower their serum cardiac troponin concentrations by changing to healthier diets, presumably mitigating the effects of subclinical cardiomyocyte injury.[28,30] Although the cause and clinical relevance of possible subclinical cardiomyocyte injury in some healthy dogs eating GF diets remain unknown, a recent report showed differences in biochemical compounds in dog foods that seem to be associated with diet-associated DCM compared to dog foods that do not appear to be associated with diet-associated DCM.[31] More information is needed to understand if these differences represent excesses or deficiencies of certain nutrients or other biochemical compounds.

Regardless of the underlying causes of observed hs-cTnI increases that might signal subclinical myocardial injury associated with the consumption of GF diets, the results of our longitudinal study importantly indicate reversibility after diet change in this group of dogs. Our results support the potential for the myocardium to recover

from diet-associated DCM, as has been described in several clinical reports.[9,11-13]

We also found a significant time: group interaction for FS. Visual inspection of the relationship between the GF dogs and GI dogs over time (Figure 3B) showed fluctuations between timepoints without clear separation of groups. The clinical importance of this finding should be verified in larger studies. Although EDVI was significantly different between groups and changed significantly over time, possibly because of measurement variability between timepoints, no significant time: group interaction was identified. We also failed to find differences in NT-proBNP between GF dogs and GI dogs over time or as a group: time interaction, although a group difference was detected. Relatively few dogs were enrolled on the basis of increased NT-proBNP concentration, and it is not surprising that this indicator of myocardial stretch in these dogs with subclinical cardiac abnormalities did not change significantly after diet intervention in either group.

A unique aspect of our study is the combined serial evaluation of cardiac biomarkers and echocardiography in both GF dogs and GI dogs that underwent diet change. The results support a cardiomyocyte injury-based mechanism underlying the development of heart disease in some dogs eating GF diets. Our study had several limitations. Small sample size and the limited number of breeds studied limit extrapolation to a larger canine population, and we likely had low statistical power to detect some clinically meaningful differences in terms of changes in NT-proBNP and some of the echocardiographic variables. One dog received taurine supplementation because of a slightly low whole blood taurine concentration. Although unlikely, we cannot exclude the possibility that this supplementation affected the results. Genetic influences (aside from the known mutations associated with DCM in Doberman Pinschers) and environmental factors for the dogs in our study are unknown, and it is possible that the current diet-associated DCM issue is multifactorial. Cardiac rhythm status was not evaluated by 24-hour Holter monitoring in our study and, therefore the effect of diet change on the dogs' rhythm status cannot be determined. Our statistical approach and the control group were used to lessen the effects of individual patient biologic variability of cardiac biomarkers, but some residual effect could have occurred. We considered it necessary to offer a small choice of intervention diets to encourage study participation and meet the needs of individual dogs. We controlled for the chosen intervention diet, but using several diets could have introduced some variable nutrient composition that might have limited the ability to detect changes in some variables. Many commercially available diets were not included, either as baseline or intervention diets, and our results generally should be viewed with a focus on the observed group differences over time, rather than specifically concentrating on certain dog foods.

Our results showed that dogs fed GF diets before study enrollment experienced decreases in serum hs-cTnI concentrations and LVIDsN over a 1-y follow-up period after diet change, compared with dogs fed GI diets before study enrollment that did not demonstrate these improvements. These findings support the potential for disease reversibility and suggest that even apparently healthy dogs eating GF diets with subclinical cardiac abnormalities might benefit from changing to a GI diet that is low in pulses and potatoes. Although increased cardiac troponin I concentrations signal cardiomyocyte injury, they do not provide information about etiology, and, therefore, future research should be directed at determining the underlying causes behind mild increases in hs-cTnI concentrations in dogs eating GF diets. Additional studies to determine how diet change might impact other breeds or dogs with overt cardiac disease also are warranted.

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## CONFLICT OF INTEREST

Dr. Adin acknowledges research support from Nestle Purina PetCare and is a consultant and sponsored lecturer for Ceva Animal Health and Boehringer Ingelheim. Dr. Freeman has received research or residency funding from, given sponsored lectures for, or provided professional services to Aratana Therapeutics, Elanco, Guiding Stars Licensing Co, LLC, Hill's Pet Nutrition, Nestlé Purina PetCare, P&G Petcare (now Mars), and Royal Canin. Dr. Rush has received funding from, given sponsored lectures for, or provided professional services to Aratana Therapeutics, Elanco, Hill's Pet Nutrition, Nestlé Purina PetCare, Royal Canin, IDEXX and Boehringer Ingelheim. Ms. Haimovitz is a student representative for Nutramax Laboratories, Inc. Drs. Goldberg and Vereb, and Ms. Lessard do not report conflicts of interest. The conflicts of interest reported by the authors did not influence data collection, data interpretation, or manuscript preparation.

## OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

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

This study was approved by the IACUC of the University of Florida, College of Veterinary Medicine (#201810504).

## HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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## SUPPORTING INFORMATION

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