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Association of diet with left ventricular wall thickness, troponin I and IGF-1 in cats with subclinical hypertrophic cardiomyopathy

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Abstract

Background: Cats with subclinical hypertrophic cardiomyopathy (sHCM) have elevated serum insulin and serum amyloid A concentrations correlating with the degree of cardiac hypertrophy. Diet might affect these and other cardiac variables.

Objective: Evaluate the effect of a complete, balanced diet with restricted starch and supplemented with eicosapentaenoic acid + docosahexaenoic acid (EPA + DHA) on echocardiographic variables and cardiac biomarkers in cats with sHCM.

Animals: Forty-four client-owned cats with sHCM.

Methods: A prospective, randomized, double-blind, multicenter study enrolled cats with end-diastole interventricular septum thickness (IVSd) or left ventricular wall thickness (LVWd) ≥6 mm, or both. Nonsedated, fasted cats were examined at baseline and after 6 and 12 months of Test (restricted starch and EPA + DHA supplements) (n = 23) or Control (unrestricted starch without EPA + DHA supplementation) (n = 21) diet. Assessments included auscultation, body weight, body condition score, echocardiography and blood analysis. Linear and generalized mixed models analyzed diet, time and diet * time interactions (5% significance level).

Abbreviations: 2D, 2-dimensional; Ao, aorta; BCS, body condition score; BW, body weight; CHF, congestive heart failure; cTnI, cardiac troponin I; CV, coefficient of variation; DHA, docosahexaenoic acid; DLVOTO, dynamic left ventricular outflow tract obstruction; EPA, eicosapentaenoic acid; HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; HR, heart rate; IGF-1, insulin-like growth factor-1; IVS, interventricular septum; IVSd, end-diastolic interventricular septum thickness; LA, left atrium/atrial; LA/Ao, ratio of the left atrial dimension to the aortic annulus dimension; LV, left ventricle/ventricular; LVFW, left ventricular free wall; LVH, left ventricular hypertrophy; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVOT, left ventricular outflow tract; LVWd, end-diastolic left ventricular free wall thickness; ME, metabolizable energy; NFE, nitrogen-free extract; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RVOT/PA, right ventricular outflow tract/pulmonary artery; SAA, serum amyloid A; sHCM, subclinical hypertrophic cardiomyopathy; T4, total thyroxine.

Results: No differences between diet groups were significant for any variable at any timepoint. There were significant decreases in the Test but not Control group in maximum IVSd (P = .03), maximum LVWd (P = .02) and insulin-like growth factor-1 levels (P = .04) after 12 months, and in ultrasensitive cardiac troponin I (cTnI) (P = .001) after 6 months; effect sizes (95% confidence interval) were 0.53 (0.09; 0.99), 0.63 (0.18; 1.09), 0.61 (0.16; 1.07), and 0.37 (−0.06; 0.8), respectively.

Conclusions and Clinical Importance: Cats with sHCM fed Test diet had significant decreases in echocardiographic variables of sHCM and in cTnI and IGF-1.

KEYWORDS

cardiac hypertrophy, cat, hypertrophic obstructive cardiomyopathy, insulin/IGF-1 mediated growth

1 | INTRODUCTION

Subclinical hypertrophic cardiomyopathy (sHCM) has a prevalence of ~15% in apparently healthy cats.[1,2] Medication of cats with sHCM remains controversial, as there is no proof of efficacy of any treatment. Drug treatment is based on extrapolation from human trials, experimental research models, anecdotes and personal opinions.[3] Current therapy targets clinical markers of cardiovascular risk and morbidity, and any effect on disease progression, morbidity or outcome remains unclear due to the absence of clinical trials.[4]

In humans, insulin plays an important role in cardiac hypertrophy through Akt-signaling pathways, stimulating protein synthesis and inhibiting protein breakdown in cardiomyocytes, and causing ventricular hypertrophy.[5] These effects are enhanced by insulin-like growth factor-1 (IGF-1), as insulin and IGF-1 can both activate each other's receptor, albeit with reduced affinity compared with their own receptors.[6] Hypertrophic cardiomyopathy (HCM) in cats has genotypical and phenotypical similarities to HCM in humans, although the disease is more severe and progresses more quickly in some cats.[7] Insulin, IGF-1 and inflammation are implicated in cats with sHCM.[8] Serum insulin concentrations are significantly higher than the laboratory reference range in cats with sHCM.[8] Serum amyloid A (SAA) levels are significantly higher in cats with generalized hypertrophy compared to cats with focal or multifocal hypertrophy, and are significantly correlated with the number of hypertrophied regions in the interventricular septum (IVS) (ρ = .28, P = .05).[8] In humans, circulating concentrations of IGF-1 are higher in patients with HCM compared with controls,[9] regional expression of IGF-1 in the myocardium is elevated,[10] and inflammation plays a role in the pathophysiology of heart failure.[11] As in humans with HCM, exogenous modulation of the insulin/IGF-1 axis and myocardial inflammation might be beneficial in the management of cardiovascular disease with increased myocardial mass in cats.[11,12]

Dietary digestible carbohydrates greatly influence the availability of insulin to the heart, and might therefore play a role in cardiomyocyte growth.[13] Omega-3 fatty acids might have effects on cardiac structure and function through their anti-inflammatory actions, as well as leading to a decrease in protein synthesis and to remodeling of hypertrophic cardiomyocytes.[14-17]

We hypothesized that dietary starch and omega-3 fatty acids influence cardiomyocyte hypertrophy and therefore thickness of the IVS and left ventricular free wall (LVFW). The objective of this study was to evaluate a complete diet with restricted starch and supplemented with eicosapentanoic acid + docosahexaenoic acid (EPA + DHA) for its effect on echocardiographic measurements and cardiac biomarkers in cats with sHCM.

2 | MATERIALS AND METHODS

2.1 | Study design

We performed a prospective randomized, double-blind, multicenter study to evaluate the effects of diet on clinical, biochemical and echocardiographic variables in cats with sHCM.

Adult cats from 8 months of age were recruited from 4 centers in the United Kingdom (A-D). Cats were enrolled with the informed consent of owners and ethical approval was obtained from the Royal Canin Ethics Committee, Institution A Committee on Research Ethics (VREC335) and Institution C Committee on Research Ethics (VERC 93.16).

To be eligible, cats had to be diagnosed with HCM on echocardiography and have no clinical signs of heart disease (International Small Animal Cardiac Health Council stage 1b).[19] Hypertrophic cardiomyopathy was diagnosed when the greatest IVS thickness at end-diastole (IVSd), or LVFW wall thickness at end-diastole (LVWd), or both measured ≥6 mm on M-dimensional or 2-dimensional (2D)-mode.[20] Additional findings consistent with HCM in some cats included systolic anterior motion of the mitral valve, dynamic LV outflow tract obstruction or left atrial enlargement.

Exclusion criteria included important concurrent systemic disease based on history, physical examination and laboratory testing (biochemistry profile and total thyroxine [T4]). Cardiac medications were allowed if they had been administered for at least 8 weeks before study start. If cardiac medication was clinically necessary after initial diagnosis of HCM, study entry was postponed for a minimum of 8 weeks.

All assessments were performed at baseline before dietary intervention, and at 6 and 12 months after the initiation of study diets. Physical examinations, body condition score (BCS on a 9-point scale[21]), blood pressure measurement and echocardiography were performed by a board-certified cardiologist or a board-eligible cardiologist supervised by a board-certified cardiologist. Blood pressure was measured by Doppler technique. The mean was taken from 3 systolic values that were within 10% of each other, selected from at least 5 serial measurements taken after acclimatization of the cat. Cats with a systolic blood pressure >180 mmHg were considered hypertensive and excluded from the study.[22]

2.2 | Echocardiography

Echocardiography (2D, M-mode and color flow, spectral, and tissue Doppler) was performed on all cats in the conscious state without chemical restraint, scanned from beneath in lateral recumbency on a purpose-designed table. A 7.5 to 12 MHz transducer was used to perform transthoracic echocardiography as recommended for cats by the Echocardiography Committee of the Specialty of Cardiology.[23] A minimum of 3 and usually 5 measurements of each echocardiographic variable were made, and the mean value determined and recorded. Echocardiographic measurements were made off-line immediately after echocardiography by the same investigator carrying out echocardiography. Values were uploaded into the study website on the same day. All cats had a full echocardiographic assessment, including assessment of diastolic function and estimation of left-side filling pressures, but only the measurements detailed were assessed as part of this study.

Right parasternal 4-chamber and 5-chamber long-axis and short-axis views at papillary muscle, mitral valve and aortic valve levels were taken. Left ventricular (LV), mitral valve, and left atrial/aortic M-modes were obtained after cursor placement guided by 2D images. M-mode measurements used leading-edge to leading-edge methodology. Two-dimensional measurements of the IVS, LV cavity and LVFW at the end of diastole and systole were made on the short-axis view at papillary muscle level, using blood-endocardial and myocardial-epicardial interfaces to guide caliper placement. Two-dimensional left atrial and aortic measurements were obtained from the right parasternal short-axis view at the end of diastole (start of QRS complex) when endocardial borders could be reliably visualized. Two-dimensional IVS and LVFW measurements were taken from the right parasternal short-axis and long-axis (4-chamber or 5-chamber) view of the LV at the end of diastole.[22] Interventricular septum in diastole was measured in basal, mid and apical regions; LVWd was measured in basal and mid regions from right parasternal long-axis 4-chamber or 5-chamber views, whichever optimized any focal hypertrophy. Papillary muscles and any false tendons were excluded from these measurements. All values were recorded as maximum thickness (max-), the sum of thicknesses measured in the 5 separate regions (sum-), and the number of regions with ≥6 mm thickness (n-). Generalized hypertrophy was defined as thickness ≥6 mm in all 5 regions of both IVSd and LVWd. Left atrial (LA) enlargement was defined as LA max ≥16 mm,[24] or short axis end-diastolic ratio of the left atrial dimension to the aortic annulus dimension (LA/Ao) ≥1.5, or both.[25]

Heart rate was calculated as 60 000/R-R interval (in milliseconds) from an average of 3 cardiac cycles in sinus rhythm, measured toward the end of right-sided echocardiography, after the cat had acclimatized. If contemporaneous electrocardiogram data were not available for heart rate, the rate counted during auscultation was used.

Left apical 5-chamber views were obtained for spectral Doppler assessments of LV outflow tract (LVOT) and aortic flow. Cranial right and left parasternal views optimizing right ventricular outflow tract/pulmonary artery (RVOT/PA) flow were obtained for spectral Doppler from these regions; measurements were made from the images which best optimized these. Velocities of LVOT, aortic and RVOT and pulmonic flow were taken from pulsed-wave envelopes (or continuous wave if aliasing was evident) in spectral Doppler recordings. In order to assist with clinical staging and prognostication for individual cats (not part of this study), diastolic function and left-sided filling pressures were evaluated,[26,27] facilitated by vagal maneuvers if required.[28]

Echocardiographic measures on stored images of LA, IVSd and LVWd from 13 cats and 4 observers from site A, and from 6 cats and 2 observers from site B were analyzed to estimate coefficients of variation (CV).

Three categories of CV were analyzed: intraobserver intracenter CV, interobserver intracenter CV, and interobserver intercenter CV. The entire echocardiographic study was provided for the second or third observer (interobserver CV) for additional measurements, so each observer could select which cine loops or images were appropriate for measurement (ie, the same stored image or loop or cardiac cycle was not necessarily remeasured for this part of the study).

2.3 | Blood analysis

Blood was collected by cephalic or jugular venipuncture into serum tubes after an overnight fast. Samples were divided into 3 aliquots, frozen at −20°C and shipped the same day to Idexx Bioanalytics, Germany. One aliquot was thawed on the day of arrival for analysis of biochemistry (Beckman Coulter AU58000, Beckman Coulter, Brea CA), total T4 (DRI Thyroxine, Microgenics, Fremont, California), cardiac troponin I (cTnI) (ADVIA Centaur TnI-Ultra Assay, Siemens Healthineers, Germany), glucose (Immulite 2000/XPi, Siemens Healthineers, Germany), IGF-1 (CLIA, Siemens, Immulite 2000, Idexx, Germany) and SAA (LAA, Eiken Chemical, Beckman Coulter AU5800, Idexx, Germany). One aliquot was kept frozen at −80°C and shipped on dry ice to the UK for analysis of insulin (IRMA, Beckman Coulter, Nationwide, UK) within approximately 8 days after blood collection. The remaining aliquot was thawed on the day of arrival or frozen at −80°C and thawed within 2 days for analysis of N-terminal pro B-type natriuretic peptide (NT-proBNP) (Feline Cardiopet NT-proBNP Immunoassay, Idexx, Germany).

2.4 | Dietary intervention

Cats were allocated to 1 of 2 coded diets (Test and Control) by a computer-based random number generator during verification of eligibility. Cardiologists, technicians, owners, and investigators were blinded to diet allocations until the final study results were analyzed and interpreted. The only investigator aware of the diet assignment was not involved in cat enrollment. There was a dry and moist format option for each diet; owners could opt for feeding 1 or a mixture of formats according to the cat's preference.

Both diets were mildly sodium-restricted (<0.7 g/MCal) and were complete and balanced (ie, sufficient to supply all nutrient requirements). Dry and moist formats for each diet had similar nutrient profiles. The main differences between Test diet and Control diet were the levels of nitrogen-free extract (% dry matter − [% protein + % fat + % crude fiber + % ash]), starch, protein, and EPA + DHA (Table 1). The Test diet was restricted in starch, higher in protein than the Control diet and supplemented with EPA + DHA. Diet rationing was based on recommended energy intake for the BCS of each cat (BCS 1-5: 78 kcal/kg bodyweight $[BW]^{0.71}$; BCS 6-9: 62 kcal/kg $BW^{0.71}$). Owners were instructed to feed study diet exclusively without addition of treats, table scraps or other food. If cats showed signs that intake was too low or too high, intake was adjusted to give 10% energy increase or decrease, respectively. Baseline assessments were repeated after 6 months and 12 months of feeding study diet.

TABLE 1 Composition of Test and Control diets

	Test diet		Control diet	
	Dry	Moist	Dry	Moist
Moisture (%)	5.5	80	5.5	79
Protein (g/MCal)	125	133.6	63.6	113.4
Fat (g/MCal)	34.8	41.3	36.8	38.5
EPA + DHA (g/MCal)	0.6	1.8	0.019	0.05
Starch (g/MCal)	38.8	19.4	113.4	46.5
NFE (g/MCal)	57.3	32.2	117.9	59.5
Sodium (g/MCal)	0.68	0.6	0.64	0.6
ME (kcal/kg)	3765	383	3955	392

Abbreviations: DHA, docosahexaenoic acid; DM, dry matter; EPA, eicosapentaenioc acid; ME, metabolizable energy; NFE, nitrogen-free extract.

2.5 | Statistical analysis

Primary endpoints were IVSd and LVWd. Secondary endpoints were LA dimension and IGF-1, insulin, NT-proBNP, and cTnI concentrations. Power analysis estimated that a total sample size of between 40 and 100 cases was required to detect a medium to large effect of diet.[29,30] All data were analyzed using the Statistical Analysis Systems Institute package (SAS version 9.4; SAS Institute Inc.). Data are expressed as median and range (minimum-maximum) unless stated otherwise.

Differences between diet groups at baseline were analyzed using unpaired *t* tests for continuous variables (age, BW, and BCS), and chi-squared tests for nominal variables (sex, medication, breeds, site, diet format, and murmur). Linear mixed models (2-way repeated-measures ANOVA comparing measured variables with visit time and group) were used for continuous variables, and generalized linear mixed models for nominal variables. Time, diet and the interaction between them were defined as fixed effects; cat was a random term. Site effect was removed from the analysis after it was shown not to have a significant effect. For linear mixed models, data were log or rank transformed if needed in order to fulfill model assumptions (normally distributed residuals and homoscedasticity). Multiple pair-wise post hoc comparisons were made for those fixed effects that were significant, and these comparisons were adjusted using Tukey honest significant difference to avoid alpha risk inflation.

Between timepoints within diet groups Cohen's *d* effect size for continuous variables and odds ratio for discrete variables were calculated with their associated 95% CI.

The level of statistical significance was set at $P < .05$, for all 2-sided analyses.

Three categories of CV (intraobserver intracenter CV, interobserver intracenter CV, and interobserver intercenter CV) were calculated with each set of observations for the corresponding category of CV ([SD/mean] * 100).

3 | RESULTS

3.1 | Study group

Between November 2015 and June 2017, 55 cats were screened for enrollment, 51 cats were eligible and received their allocated diet and 44 completed the study (Figure 1). One cat in the Test group died suddenly following vaccination 4 months after study start; it was assumed to have had acute cardiac failure. There were 2 deaths in the Control group: 1 at 8 months and 1 at 6 months after study start due to chronic heart failure and unknown cause, respectively. Results are presented for the 44 cats that completed the study. The Test group consisted of 23 cats (dry diet n = 10, moist diet n = 2 and mixed dry and moist diet n = 11) and the Control group consisted of 21 cats (dry diet n = 4, moist diet n = 2 and mixed dry and moist n = 15) (Table 2). Cats ate the same diet formats throughout the study. Over the 12-month study period, there were no reports of diet refusal or changed eating behavior, and BW or BCS did not significantly change (Table 3).

There were no significant differences at baseline between Test and Control group in signalment (Table 2), clinical characteristics (Table 2), medical center distribution (Table 2), biochemical variables (Table 3), or echocardiographic measurements (Table 4). The median age across groups was 5 (range, 1.4-17) years. Most cats (70%) were

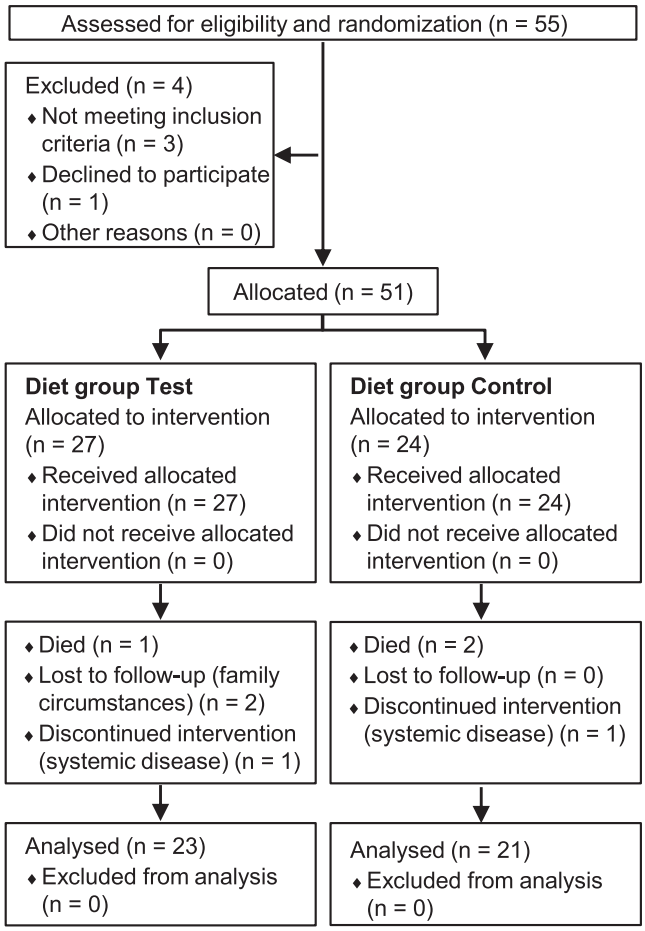

FIGURE 1 Consolidated Standards of Reporting Trials (CONSORT) flow diagram[18] detailing the number of cats that were randomized and assessed for eligibility, that were allocated a diet and that completed the 12 month dietary intervention. For practical reasons, cats were allocated to 1 of 2 diet groups by a computer-based random number generator during verification of eligibility. After ensuring eligibility, cats were enrolled and received the allocated diet

male. Forty-two cats (95.5%) had a murmur and this was grade ≥3 in 28 (63.6%) cats. Median (range) blood pressure was 130 (92-165) mm Hg at baseline, 130 (94-162) mm Hg after 6 months, and 122 (96-180) mm Hg after 12 months.

Eight cats received medications (atenolol with or without clopidogrel or telmisartan) (Table 2). No cats initiated medication during the study and no cats on medication at baseline stopped medication.

3.2 | Primary and secondary endpoints

There was a significant diet * time interaction for both IGF-1 (P = .02) and heart rate (P = .02). IGF-1 significantly decreased between baseline and 12 months in the Test group compared with no difference in the Control group (effect size [95% CI] 0.61 [0.16; 1.07] vs −1 [−0.54; 0.33]) (Table 4, Figure 2). Heart rate significantly decreased between baseline and 12 months in the Test group (P = .005), but was not significantly different between the 2 groups at 12 months (effect size [95% CI] 0.65 [0.19; 1.13] for the Test group and 0.31 [−0.14; 0.76] for the Control group) (Table 4).

Several other variables significantly changed over time in the Test group but not in the Control group; however no differences between the diet groups were significant for any variable at any timepoint. There was a significant effect of time for primary endpoints max-IVSd (P = .004), max-LVWd (P = .004), n-IVSd (P < .001) and sum-IVSd (P < .001) (Figure 2). All of these variables were significantly decreased at 12 months in the Test group but not the Control group. Effect sizes (95% CI) between baseline and 12 months were higher for the Test group compared with the Control group: 0.53 (0.09; 0.99) vs 0.36 (−0.09; 0.82) for max-IVSd, 0.63 (0.18; 1.09) vs 0.22 (−0.22; 0.67) for max-LVWd, 0.86 (0.38; 1.36) vs 0.44 (−0.02; 0.9) for n-IVSd and 0.87 (0.39; 1.38) vs 0.58 (0.12; 1.07) for sum-IVSd. Median max-LVWd decreased in the Test group after 12 months to values below cut-off level of 6 mm (Table 4).

Secondary endpoints and other variables are described in Tables 3 and 4. In the Test group, cTnI decreased significantly between baseline and 6 months but not 12 months (effect size [95% CI] 0.37 (−0.06; 0.8) and 0.33 (−0.1; 0.76), respectively), compared with no significant change in the Control group at either timepoint (Table 3, Figure 2). Although there were no significant differences between diet groups, normalization of both max-IVSd and max-LVWd (≤6 mm wall thickness) occurred after 12 months of diet in 30% of cats in Test group and 14% of cats in Control group.

3.3 | Subpopulations

To evaluate the effect of the diet in cats with more or less advanced sHCM, a subanalysis was conducted on cats with and without LA enlargement.

In cats without LA enlargement, there was a significant effect of time and a significant decrease between baseline and 12 months in the Test group for n-IVSd, sum-IVSd, max-LVWd, LA-max, and heart rate (Table 5). Time also had a significant effect on max-IVSd, sum-LVWd, n-(IVSd + LVWd), and cTnI, but decreases in these values at 12 months were not significant in either diet group (Table 5).

In cats with LA enlargement, the effect of time on n-IVSd and sum-IVSd was significant, as was the decrease in these variables between baseline and 12 months in the Test group. There was a time effect for n-(IVSd + LVWd), max-LVOT, max-RVOT, and cTnI, without significant changes in these variables from baseline to 12 months (Table 5). However, in the Test group but not the Control group there were significant decreases at 6 months in max-LVOT (P = .02 and P = .98, respectively) and cTnI (P = .03 and P = .99, respectively), with effect sizes (95% CI) of 0.86 (0.21; 1.55) for max-LVOT and 0.41 (−0.17; 1.01) for cTnI.

TABLE 2 Baseline demographics, clinical characteristics, diet format, and center distribution of study cats with subclinical hypertrophic cardiomyopathy

Variable	Test group (n = 23)	Control group (n = 21)	*P* value
Age (years)	4.3 (1.42-17)	7.9 (2-13.9)	.47
Sex			.6
Male	17	14	
Female	6	7	
Body weight (kg)	5.0 (2.9-8.7)	4.8 (3.2-7.5)	.7
Body condition score (1-9)	5 (3-9)	5 (4-7)	.55
Medications (n)	2	6	.08
Atenolol	2	3	
Atenolol and telmisartan	0	1	
Atenolol and clopidogrel	0	2	
Breeds (n)			.06
DSH + DLH	19	12	
British Shorthair	0	3	
Selkirk Rex	1	2	
Persian	1	1	
Ragdoll cross	1	1	
Exotic shorthair	1	0	
Siamese	0	1	
Maine Coon	0	1	
Centers (n)			.83
A	12	12	
B	9	8	
C	1	1	
D	1	0	
Diet format (n)			.2
Dry only	10	4	
Wet only	2	2	
Mixed	11	15	
Heart murmur (n)	23	19	.14
Grade 1-2	6	8	
Grade ≥ 3	17	11	

Note: Data are presented as median and range (minimum-maximum).
Abbreviations: DLH, domestic long-hair; DSH, domestic short-hair.

3.4 | Intraobserver and interobserver variability

There were 3 categories of CV: intraobserver intracenter CV ranging from 3.2% to 5.2%, interobserver intracenter CV ranging from 3.7% to 10.7%, and interobserver intercenter CV ranging from 4.2% to 18.9% (Table 6). Intraobserver and interobserver CV for primary endpoints max-IVSd and max-LVWd were ≤5.9%.

4 | DISCUSSION

The results of this study support for the first time an effect of diet on echocardiographic variables in cats with sHCM. Cats fed Test diet for 12 months had significantly decreased thickness of IVSd and LVWd, with the latter measurements being comparable to healthy cats. There were also significant reductions in cTnI and IGF-1, with the latter interacting significantly with diet. These findings are consistent with a previous study in which insulin, IGF-1 metabolism and inflammation were identified as possible contributors to the mechanism modulating sHCM.[8] The Test diet was formulated with the objective of minimizing basal and peak insulin concentrations in the blood, and it was supplemented with fish oil, known for its anti-inflammatory properties. Exposure of the heart to insulin and IGF-1 is mainly determined by dietary intake of digestible carbohydrates.[13] Low carbohydrate diets have proven effectiveness in improving cardiac remodeling and function in humans and rats.[13,31,32]

Despite significant changes from baseline in primary and secondary endpoints in the Test group, the lack of significant

TABLE 3 Body weight, body condition score, and laboratory measurements in cats with subclinical hypertrophic cardiomyopathy randomized to Test or Control diet for 12 months

	Test group (n = 23)			Control group (n = 21)		
	Baseline	**6 months**	**12 months**	**Baseline**	**6 months**	**12 months**
BW (kg)	5.0 (2.9-8.7)	4.9 (2.7-7.9)	4.9 (2.7-7.7)	4.8 (3.2-7.5)	4.9 (3.28-7.8)	4.9 (3.2-7.4)
Effect size (95% CI)		0.09 (−0.33; 0.51)	0.28 (−0.14; 0.71)		0.11 (−0.33; 0.55)	0.21 (−0.23; 0.66)
BCS (1-9)	5 (3-9)	6 (2-8)	6 (2-7)	5 (4-7)	5 (3-8)	5 (3-7)
Effect size (95% CI)		−0.06 (−0.48; 0.36)	0.15 (−0.27; 0.57)		0.06 (−0.38; 0.5)	0.15 (−0.29; 0.59)
Insulin (μU/mL)	24 (8.9-113)	32.5 (6.6-58)	42 (6.3-70)	16.7 (3.9-76)	24.2 (7.4-123)	16.8 (6.2-129)
Effect size (95% CI)		−0.33 (−0.76; 0.1)	−0.49 (−0.94; −0.05)		−0.36 (−0.82; 0.09)	−0.18 (−0.63; 0.26)
IGF-1 (ng/mL)	460 (216-772)	369 (118-658)	**401 (73.3-720)**[a]	448 (207-786)	463 (66.5-956)	498 (132-1000)
Effect size (95% CI)		0.88 (0.4; 1.38)	0.61 (0.16; 1.07)		−0.05 (−0.49; 0.39)	−0.1 (−0.54; 0.33)
SAA (mg/L)	0 (0-1.5)	0 (0-34.5)	0 (0-1.3)	0.0 (0.0-21.8)	0.05 (0-12.4)	0 (0-34)
Odds ratio (95% CI)		1.00 (0.26; 3.81)	0.43 (0.08; 1.87)		2.20 (0.64; 7.96)	1.23 (0.35; 4.46)
NT-proBNP (pmol/L)	211 (24-1500)	98 (24-1500)	150 (26-1500)	271 (26-1500)	199.5 (24-1500)	263 (24-1500)
Effect size (95% CI)		0.14 (−0.28; 0.56)	−0.11 (−0.53; 0.31)		−0.09 (−0.53; 0.35)	−0.39 (−0.85; 0.06)
cTnI (ng/mL)	0.16 (0.02-9.49)	**0.07 (0.01-0.55)**[b]	0.12 (0.01-.56)	0.14 (0.01-1.74)	0.1 (0.01-2.25)	0.1 (0.01-2.35)
Effect size (95% CI)		0.37 (−0.06; 0.8)	0.33 (−0.1; 0.76)		0.02 (−0.41; 0.46)	−0.23 (−0.68; 0.21)

Note: Data are presented as median and range (minimum-maximum). There was no significant difference between diet groups at baseline. Text in bold indicates statistically significant differences for other timepoints compared with baseline.
Abbreviations: CI, confidence interval; BCS, body condition score; BW, bodyweight; cTNI, cardic troponin I; IGF-1, insulin-like growth factor-1; NT-proBNP, N-terminal pro B-type natriuretic peptide; SAA, serum amyloid A.
[a]$P = .04$ and [b]$P = .001$ for the comparisons with baseline of the Test group. Effect sizes (Cohen's *d*) between baseline and the stated timepoint are given for each variable within each diet group; SAA was analyzed as discrete (nominal) data and therefore odds ratios are shown instead.

difference between the diets at any timepoint for any variable, limits clinical interpretation of the findings. The absence of statistical differences between diets with respect to IVSd and LVWd might be explained by the fact that these variables declined non-significantly in the Control group as well as declining significantly in the Test group, although the effect sizes were larger in the Test group.

There was a significant diet * time interaction for IGF-1, and IGF-1 also decreased significantly with regression of IVSd and LVWd hypertrophy over 12 months in cats fed Test diet. Cats with hypersomatotropism frequently have ventricular hypertrophy.[33,34] Our findings are comparable with the reversible effect of hypophysectomy on cardiac hypertrophy and the concurrent decrease in circulating IGF-1 in cats with hypersomatotropism.[35] However, the main effect of diet on insulin/IGF-1 metabolism was decreased IGF-1 and there was no effect on insulin concentrations. This is surprising, considering that cats with sHCM have insulin concentrations elevated above the laboratory reference range,[8] similar to those reported in humans.[36,37] Possibly a relatively high protein and amino acid content in our Test diet prevented a reduction in insulin secretion.[38] It is also possible that the overnight fasting before blood sampling was too short for decreases in basal insulin concentrations to be observed. In rats, the hypertrophic response of cardiac myocytes to insulin is greater than that to IGF-1.[39] Our data suggest that in cats, reversal of cardiac hypertrophy can occur with decreases in IGF-1 levels without concurrent decreases in insulin levels.

There was also a similar significant diet * time interaction for heart rate, which decreased over time, but only in the Test diet group. This observation remains unexplained—if it reflected acclimatization of cats to study procedures the same effect would be expected in the Control group. It is unlikely that heart rate reduction affected the primary endpoints of diastolic wall thickness or LA dimension, especially as there was no significant difference in heart rate between groups at each time point and the rate reduction in the Test group was minor (median reduction 4 bpm). Although heart rate (R-R interval) can influence variables associated with diastolic function[27] and systolic function, no major effect on LVFW thickness or left atrial size is expected[26] or has been reported in cats with sHCM.[40] In anesthetized healthy cats, there is a significant correlation between diastolic wall thickness and heart rate when atrial pacing is used to manipulate heart rate.[41] Whether the converse is true in this study with unsedated cats with physiological heart rates is unclear.

We also investigated the effect of omega-3 fatty acids on the inflammatory marker SAA. Omega-3 fatty acids have beneficial effects in cardiovascular disease, for managing arrhythmias, but also myocardial energy metabolism, endothelial and immune function, heart rate, and blood pressure.[17] With respect to cardiac remodeling, in vitro EPA + DHA treatment of rat hypertrophic cardiomyocytes decreases protein synthesis, cell surface area, atrial natriuretic peptide levels and remodeling.[15,16,42] A previous study showed that SAA levels were not significantly higher than the laboratory reference range in cats with sHCM, although they were higher in cats with generalized hypertrophy compared to focal or

TABLE 4 Echocardiographic measurements in cats with subclinical hypertrophic cardiomyopathy randomized to Test or Control diet for 12 months

	Test group (n = 23)			Control group (n = 21)		
	Baseline	**6 months**	**12 months**	**Baseline**	**6 months**	**12 months**
Heart rate (bpm)	169 (140-240)	NA	**165 (101-187)**[a]	171 (118-220)	NA	165 (131-204)
Effect size (95% CI)			0.65 (0.19; 1.13)			0.31 (−0.14; 0.76)
2D assessments (mm)						
Max-IVSd	6.97 (5.50-8.70)	6.50 (5.00-7.90)	**6.30 (3.93-9.00)**[a,b]	7.30 (5.90-9.20)	6.80 (5.40-8.90)	6.70 (4.30-9.90)
Effect size (95% CI)		0.34 (−0.09; 0.77)	0.53 (0.09; 0.99)		0.4 (−0.05; 0.86)	0.36 (−0.09; 0.82)
n-IVSd	2.0 (0.0-3.0)	2.0 (0.0-3.0)	**1.0 (0.0-3.0)**[a,b,c,d]	2.0 (0.0-3.0)	2.0 (0.0-3.0)	2.0 (0.0-3.0)
Effect size (95% CI)		0.32 (−0.1; 0.76)	0.86 (0.38; 1.36)		0.05 (−0.39; 0.49)	0.44 (−0.02; 0.9)
Sum-IVSd	18.80 (14.40-22.50)	18.30 (13.10-21.30)	**16.50 (11.00-21.20)**[a,b]	18.80 (15.60-23.50)	19.10 (13.90-24.00)	17.70 (12.60-26.10)
Effect size (95% CI)		0.6 (0.15; 1.06)	0.87 (0.39; 1.38)		0.39 (−0.06; 0.85)	0.58 (0.12; 1.07)
Max-LVWd	6.10 (4.10-8.00)	**5.60 (3.30-7.50)**[e]	**5.30 (4.08-7.20)**[a]	6.60 (4.40-7.80)	6.10 (3.50-9.00)	6.30 (4.00-8.00)
Effect size (95% CI)		0.89 (0.41; 1.4)	0.63 (0.18; 1.09)		0.28 (−0.17; 0.73)	0.22 (−0.22; 0.67)
n-LVWd	1.0 (0.0-2.0)	0 (0.0-2.0)	0 (0.0-2.0)	1.0 (0.0-2.0)	1.0 (0.0-2.0)	1.0 (0.0-2.0)
Odds ratio (95% CI)		0.23 (0.06; 0.76)	0.41 (0.12; 1.33)		0.67 (0.19; 2.33)	0.81 (0.22; 2.89)
Sum-LVWd	11.80 (7.60-15.20)	10.70 (6.40-14.60)	10.30 (6.86-14.40)	12.11 (8.50-15.00)	11.50 (6.80-17.90)	11.70 (7.90-15.50)
Effect size (95% CI)		0.7 (0.24; 1.18)	0.54 (0.09; 0.99)		0.29 (−0.15; 0.74)	0.2 (−0.24; 0.64)
n-(IVSd + LVWd)	3.0 (1.0-5.0)	2.0 (0.0-5.0)	1.0 (0.0-5.0)	3.0 (0.0-5.0)	3.0 (0.0-5.0)	3.0 (0.0-5.0)
Effect size (95% CI)		0.53 (0.09; 0.99)	0.68 (0.23; 1.16)		0.3 (−0.14; 0.75)	0.38 (−0.07; 0.84)
LVIDs	7.40 (3.10-10.40)	7.20 (1.80-10.80)	7.70 (1.70-11.00)	7.60 (3.00-12.00)	7.50 (2.30-10.40)	6.70 (3.20-14.00)
Effect size (95% CI)		0.01 (−0.41; 0.43)	−0.19 (−0.62; 0.23)		0.18 (−0.26; 0.63)	0.3 (−0.15; 0.75)
LVIDd	14.30 (9.20-17.30)	14.30 (11.40-18.10)	15.70 (12.2-19.30)	14.20 (1.27-20.00)	14.70 (10.00-18.39)	14.10 (10.30-20.00)
Effect size (95% CI)		−0.16 (−0.59; 0.25)	−0.35 (−0.78; 0.08)		−0.41 (−0.87; 0.04)	−0.31 (−0.77; 0.13)
LA-max	15.30 (11.40-20.00)	15.40 (12.10-19.50)	16.30 (11.65-24.9)	16.00 (12.20-23.40)	16.41 (11.30-24.00)	15.50 (12.20-32.30)
Effect size (95% CI)		−0.24 (−0.66; 0.18)	−0.4 (−0.84; 0.03)		−0.26 (−0.71; 0.18)	−0.33 (−0.79; 0.11)
LA/Ao	1.27 (0.97-1.70)	1.30 (1.00-1.50)	1.30 (1.00-2.00)	1.28 (1.00-2.33)	1.30 (0.86-1.89)	1.37 (0.76-2.14)
Effect size (95% CI)		−0.21 (−0.63; 0.21)	−0.33 (−0.76; 0.1)		−0.03 (−0.46; 0.41)	−0.23 (−0.68; 0.21)
M-Mode variable (mm)						
IVSd	6.00 (3.30-8.40)	5.50 (3.20-7.60)	5.80 (2.90-6.90)	5.60 (4.10-8.70)	5.50 (3.60-7.40)	6.00 (3.00-8.41)
Effect size (95% CI)		0.67 (0.21; 1.14)	0.38 (−0.05; 0.81)		0.26 (−0.18; 0.71)	0.08 (−0.36; 0.52)
LVWd	6.40 (4.00-9.30)	5.70 (4.30-8.40)	6.10 (3.60-8.90)	6.70 (4.30-14.20)	6.50 (2.60-9.00)	6.3 (4.27-10.90)
Effect size (95% CI)		0.37 (−0.06; 0.81)	0.37 (−0.06; 0.81)		0.25 (−0.19; 0.7)	0.33 (−0.12; 0.78)
Doppler (m/s)						
Max-LVOT	2.20 (0.70-5.10)	1.38 (0.73-4.55)	1.31 (0.57-4.84)	1.46 (0.72-5.60)	1.30 (0.70-5.20)	1.51 (0.69-5.20)
Effect size (95% CI)		0.54 (0.1; 1)	0.31 (−0.11; 0.74)		0.14 (−0.3; 0.58)	−0.1 (−0.54; 0.34)
Max-RVOT	0.90 (0.00-2.60)	0.93 (0.69-1.31)	0.85 (0.66-1.99)	0.95 (0.60-2.32)	0.93 (0.49-2.24)	0.92 (0.50-2.77)
Effect size (95% CI)		0.01 (−0.4; 0.43)	0.08 (−0.33; 0.5)		−0.03 (−0.47; 0.41)	−0.05 (−0.49; 0.39)

Note: Data are shown for baseline and at the 6-month and 12-month recheck visits. Data are presented as median and range (minimum-maximum). There was no significant difference between the 2 diet groups at baseline. Effect sizes (Cohen's *d*) between baseline and the stated timepoints are given for each variable within each diet group; n-LVWd was analyzed as discrete (nominal) data and therefore odds ratios are shown instead. Results expressed in bold are significant for at least 1 comparison.

Abbreviations: CI, confidence interval; IVSd, interventricular septum thickness at end-diastole; LA/Ao, ratio of the left atrial dimension to the aortic annulus dimension; LA-max, maximum left atrial dimension; LVIDd, left ventricular internal dimension at end-diastole; LVIDs, left ventricular internal dimension at end-systole; LVWd, left ventricular free wall thickness at end-diastole; max-IVSd, maximum interventricular septum thickness at end-diastole; max-LVOT, maximum left ventricular outflow tract; max-LVWd, maximum left ventricular free wall thickness at end-diastole; max-RVOT, maximum right ventricular outflow tract; NA, not available; n-(IVSd + LVWd), number of regions in which the end-diastolic interventricular septum plus the left ventricular free wall was ≥6 mm thick; n-IVSd, number of regions in which the end-diastolic interventricular septum was ≥6 mm thick; n-LVWd, number of regions in which the end-diastolic left ventricular free wall was ≥6 mm thick; sum-IVSd, sum of end-diastolic interventricular septum thicknesses measured in 5 separate regions; sum-LVWd, sum of end-diastolic left ventricular free wall thicknesses measured in 5 separate regions.

[a]Comparisons between 12 months of the Test group and baseline of the Test group: $P = .005$ for heart rate, $P = .03$ for max-IVSd, $P < .001$ for n-IVSd, $P < .001$ for sum-IVSd, and $P = .02$ for max-LVWd.

[b]Comparisons between 12 months of the Test group and baseline of the Control group: $P = .01$ for max-IVSd, $P = .02$ for n-IVSd and $P = .009$ for sum-IVSd.
[c]$P = .04$ for the comparison of n-IVSd between 12 and 6 months of the Test group.
[d]$P = .03$ for the comparison of n-IVSd between 12 months of the Test group and 6 months of the Control group.
[e]$P = .03$ for the comparison of max-LVWd between 6 months and baseline of the Test group. *P*-values for were adjusted by Tukey honest significant difference.

multifocal hypertrophy, and were associated with the number of hypertrophied regions in the IVS.[8] Possibly therefore, inflammation was only local with limited monocyte activation. In the study described here, there was no significant effect of Test diet on serum SAA. This does not exclude an effect of EPA + DHA supplementation on local inflammation and reversal of cardiac hypertrophy. We selected SAA as a marker because our previous data showed it might be affected by the degree of hypertrophy in sHCM, but there are a range of other inflammatory markers that might have given positive results in the current study.[8]

The effect of diet in reducing cardiac hypertrophy was much less evident in cats with LA enlargement. In these cats, only n-IVSd and sum-IVSd, and not maximum cardiac thickness, decreased significantly at 12 months, while the decrease in cTnI was only significant at 6 months. Left atrial enlargement has been associated with shorter survival in cats with HCM[43] and is indicative of a more advanced stage of sHCM close to decompensation.[19] Our data suggest that when this occurs, cardiac myocytes become less sensitive to changes in insulin/IGF-1. This is not surprising, since at this stage a degree of endomyocardial or interstitial fibrosis is likely to be present.[20,44-46]

Standard evaluation of ventricular wall thickness on echocardiography uses the maximum measured value in any region of the IVS or LV wall.[47] The Test group had significant changes in the primary endpoints IVSd and LVWd, especially for the maximum thicknesses. Further characterization of the cardiac effects of Test diet were provided by less conventional evaluations, which showed that the number of areas affected as well as the sum of thicknesses measured in these areas decreased in the Test group.

Our study did not extend for long enough to ascertain whether decreased LV hypertrophy resulted in better clinical outcomes, slower disease progression or longer survival; this was beyond the remit of the study. Presence of extreme hypertrophy (≥9 mm) is a proven independent predictor of cardiac mortality in cats with HCM, with similar survival times for cats with diastolic LV wall hypertrophy between 6.0 and 8.9 mm.[47] In our study, median LVWd after 12 months of Test diet was below the diagnostic cut-off value for ventricular hypertrophy (6 mm). Whether this regression in wall thickness in the Test diet will influence clinical outcome is unknown. This needs to be investigated in a longer-term follow-up study. Interestingly, cTnI decreased significantly in the Test group over 6 months, but not in the Control group. Circulating cardiac troponins like cTnI are sensitive markers of cardiomyocyte injury,[48] and cTnI is prognostic for cardiac death in all stages of HCM.[49] Cardiac TnI concentration is reported to vary by BW,[50] but there was no difference in BW between our groups, and no significant change in BW. We propose that the decrease in cTnI is likely to have been caused by a decrease in cardiomyocyte injury.

Measurements of left ventricular wall thickness decreased in cats fed Test diet. We considered other factors such as precision of echocardiographic variables and the role of medication that might have influenced this reduction in wall thickness. The number of cats that received medication was very small and did not differ significantly between the Test and Control groups. Although numerically more cats in the Control group received medication, including atenolol, telmisartan and clopidogrel, these drugs have not been reported to reduce left ventricular wall thicknesses or affect HCM progression in cats with HCM.[51] Bodyweight did not appear to affect the results, as this was not different between the groups at baseline and did not change with reductions in ventricular wall thickness. A small effect of BW on echocardiographic measurements has been suggested in cats, and our threshold of ≥6 mm for defining hypertrophy might have masked a possible effect of BW, because larger cats can have larger hearts without underlying pathology.[40] It is unlikely however that such cats would respond to a dietary intervention with decreased cardiac hypertrophy and cardiac markers of disease. Moreover, cats with sHCM have been reported to have cardiac marker levels significantly higher than laboratory reference ranges and that were associated with measures of HCM, thereby making an effect of physiological variation in heart size in sHCM less likely.[8] Systemic hypertension might result in concentric LV hypertrophy, and this was an exclusion criterion for this study. In cats with significant dynamic left ventricular outflow tract obstruction, the increased afterload is a stimulus for further concentric left ventricular hypertrophy, and reduction in severity of obstruction might explain regression in left ventricular wall thickness over time. However, in this study, there was no significant change over time in the peak LVOT or aortic velocities in either the Test diet or Control diet groups. The presence and severity of dynamic left ventricular obstruction in cats will vary with level of stress and excitement of the patient, medications and left ventricular systolic function.

Our study had several strengths. The study had a double-blind, prospective randomized design and included multiple centers. The diets compared were noncommercial diets with the same nutrient profile and minimal differences in ingredients, and were fed for 12 months. A previous study tested the hypothesis that diet could modify clinical, biochemical or echocardiographic variables in cats with sHCM, by feeding 6 commercial diets with varying carbohydrate, fat and main ingredients.[22] Failure to show changes in IVSd and LVWd might have been due to small sample size (29 cats), a relative short duration of the treatment (6 months), or excessively large differences in nutrient profiles and ingredients between the diets. The significant decreases in IVSd, LVWd, and cTnI in the study described here

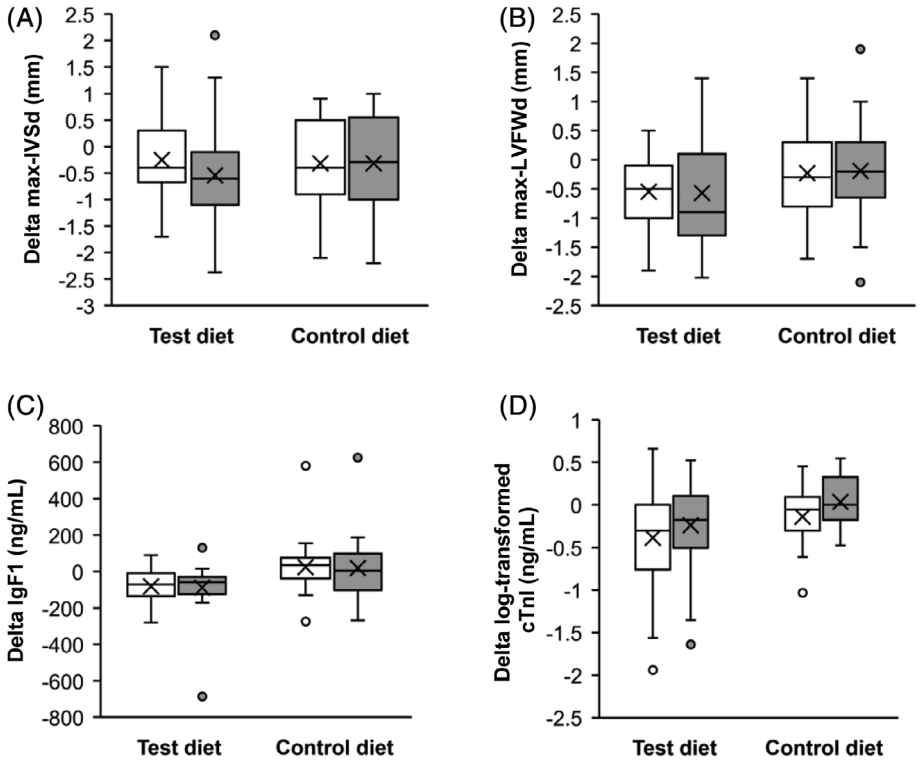

FIGURE 2 Box and whisker plots for the change (delta) in echocardiography variables from baseline to 6-month (clear boxes) and 12-month (shaded boxes) evaluations in cats with subclinical hypertrophic cardiomyopathy fed Test (n = 23) or Control diet (n = 21) for a total of 12 months. The horizontal bars represent the median values and the crosses the mean values. Whiskers show the range of values that are up to 1.5 times larger than the third quartile or 1.5 times lower than the first quartile. Circles are outliers outside of these ranges. A, Change in max-IVSd (mm). B, Change in max-LVFWd (mm). C, Change in IGF-1 (ng/mL). D, Change in log-transformed cTnI (ng/mL) (log-transformed in order to fulfill model assumptions of normally distributed residuals and homoscedasticity). The circle for the Control diet in plot D is 2 superimposed outliers with values indistinguishable on the scale presented. cTnI, cardiac troponin I; IGF-1, insulin-like growth factor-1; IVSd, interventricular septum thickness at end-diastole; LVFWd, left ventricular free wall thickness at end-diastole

suggest that the progression of sHCM in cats could possibly be slowed by reducing levels of insulin, IGF-1, and inflammation. Even though there were no significant differences between groups after 6 or 12 months of feeding study diets, the effect of Test diet on cardiac measures might be regarded as significant in a targeted population. Signalment and baseline clinical characteristics were not different between the groups, and cats in the Test group were similar to HCM populations previously described in terms of sex ratio, median age, age ranging from young adult to senior, median BW and predominant breed (domestic short-hair and long-hair breeds).[43,52,53]

A number of limitations in our study design and analysis might have impacted the findings and should be considered in their interpretation. None of the cats remained on the same diet that they were eating before the start of the study. We considered it preferable to make a direct comparison between the effects of a Test diet with a matched, study-formulated Control diet, in order to avoid potentially confounding factors of variable diet type, especially given prior data showing no significant effect of commercial diets on wall thickness.[22] Also, in Europe, regulations required that we provided both Test and Control cats with a diet appropriate for sHCM. Such a diet should be complete and balanced and made from premium ingredients. Moreover it must comply with European Commission Regulation 2020/354 on restricted sodium levels in diets to support heart function in chronic cardiac insufficiency.[54] Due to this limitation in study design, we cannot exclude the possibility of a difference between the Test and Control groups had the Control cats remained on the diets they were fed before study start. Nevertheless, although there are few serial data on changes in wall measurements over time in cats with sHCM, reduction in left ventricular wall thickness is not an expected outcome. Therefore, we might assume that if cats in the Control group had remained on their prestudy diets, they would have continued to show disease progression, but we cannot prove this. In controlled pharmacologic studies evaluating the effect of angiotensin converting enzyme inhibitors there are no changes in ventricular hypertrophy in cats receiving standard therapy.[55,56] Conversely, end-stage HCM in some cats might be associated with LV chamber dilatation, systolic dysfunction, and relative thinning of the LV walls,[57-59] but these changes are evident over years. Another condition which might result in resolution of left ventricular hypertrophy is transient myocardial thickening (TMT), which has shown fast reverse remodeling in a median of 3.3 months after antecedent events in cats that were significantly younger than HCM controls (median 1.7 years vs 8 years, respectively). The age, history

TABLE 5 Statistical significance and effect sizes for changes over time in echocardiographic measurements and cardiac troponin I in cats with subclinical hypertrophic cardiomyopathy with or without left atrial enlargement at baseline

	Cats without LA enlargement at baseline (n = 21)			Cats with LA enlargement at baseline (n = 23)		
Variable	Effect of time *P* value	Test group decrease between baseline and 12 months	Control group decrease between baseline and 12 months	Effect of time *P* value	Test group decrease between baseline and 12 months	Control group decrease between baseline and 12 months
Max-IVSd						
P value	.005	.06	.38	.38	.69	1
Effect size (95% CI)		0.92 (0.2; 1.69)	0.58 (−0.11; 1.31)		0.31 (−0.29; 0.92)	0.16 (−0.46; 0.78)
n-IVSd						
P value	.01	.04	.76	.005	.02	.81
Effect size (95% CI)		1.16 (0.38; 2.01)	0.39 (−0.28; 1.08)		0.66 (0.02; 1.34)	0.54 (−0.11; 1.22)
Sum-IVSd						
P value	.003	.02	.43	.004	.03	.37
Effect size (95% CI)		1.04 (0.3; 1.86)	0.54 (−0.15; 1.26)		0.72 (0.07; 1.4)	0.61 (−0.05; 1.3)
Max-LVWd						
P value	.02	.05	.1	.15	.56	.94
Effect size (95% CI)		0.74 (0.06; 1.47)	0.36 (−0.3; 1.05)		0.5 (−0.12; 1.14)	0.22 (−0.41; 0.85)
Sum-LVWd						
P value	.05	.11	.99	.19	.67	.98
Effect size (95% CI)		0.59 (−0.07; 1.28)	0.41 (−0.26; 1.1)		0.46 (−0.15; 1.09)	0.17 (−0.45; 0.8)
n-(IVSd + LVWd)						
P value	.02	.06	.82	.04	.15	.83
Effect size (95% CI)		0.84 (0.14; 1.59)	0.36 (−0.3; 1.05)		0.55 (−0.07; 1.2)	0.38 (−0.25; 1.03)
LA-max						
P value	.003	.01	.74	.63	1	1
Effect size (95% CI)		−1.06 (−1.88; −0.31)	−0.59 (−1.32; 0.1)		−0.22 (−0.82; 0.38)	−0.29 (−0.93; 0.34)
Max-LVOT						
P value	.95	1	1	.02	.28	.98
Effect size (95% CI)		0.08 (−0.54; 0.7)	0.02 (−0.63; 0.68)		0.5 (−0.12; 1.14)	−0.17 (−0.8; 0.45)
Max-RVOT						
P value	.54	.72	1	.01	.79	.38
Effect size (95% CI)		0.13 (−0.49; 0.76)	−0.19 (−0.85; 0.47)		0.05 (−0.54; 0.64)	0.12 (−0.5; 0.74)
HR						
P value	.006	.008	.9	.92	.58	.69
Effect size (95% CI)		0.89 (0.14; 1.7)	0.48 (−0.2; 1.18)		0.44 (−0.17; 1.07)	0.08 (−0.54; 0.71)
cTnI						
P value	.03	.91	.99	.04	.18	.92
Effect size (95% CI)		0.27 (−0.36; 0.91)	−0.03 (−0.68; 0.63)		0.41 (−0.2; 1.03)	−0.36 (−1.01; 0.27)

Note: Statistical analysis was by linear mixed models (2-way repeated measures ANOVA with the measured variables compared with both time and diet group) for continuous variables and generalized linear mixed models for nominal variables. Time, diet and the interaction between them were defined as fixed effects; cat was a random term. Statistical significance was set at $P < .05$ for 2-sided analyses. *P*-values for comparisons in variables between baseline and 12 months were adjusted by Tukey honest significant difference. Effect sizes (Cohen's *d*) between baseline and the stated timepoint are given for each variable within each diet group.

Abbreviations: CI, confidence interval; cTnI, cardiac troponin I; HR, heart rate; LA-max, maximum left atrial dimension; max-LVOT, maximum left ventricular outflow tract; max-IVSd, maximum interventricular septum thickness at end-diastole; max-RVOT, maximum right ventricular outflow tract; M-IVSd, M-mode left ventricular thickness at end-diastole; n-(IVSd + LVWd), number of regions in which the end-diastolic interventricular septum plus the left ventricular free wall was ≥6 mm; n-IVSd, number of regions in which the end-diastolic interventricular septum was ≥6 mm; sum-IVSd, sum of end-diastolic interventricular septum thicknesses measured in 5 separate regions; sum-LVWd, sum of end-diastolic left ventricular free wall thicknesses measured in 5 separate regions.

and time-course of the echocardiographic changes of the cats in this study are not consistent with TMT.

Diet history was not recorded at enrollment. Both Test and Control diets were complete and balanced with restricted sodium levels and premium ingredients. It is possible that switching to a diet of better quality and a different sodium level in the study caused the changes observed in cardiac variables and resulted in our inability to detect differences between diets.

The inclusion criterion for systolic blood pressure <180 mm Hg was higher than currently recommended[60] and it is possible that some borderline hypertensive or mildly hypertensive cats were included, but at low risk for target organ damage.[60] We selected a higher cut-off to allow for situational (stress-associated) hypertension in some cats. The population median blood pressure was normal at 130 mm Hg, so systemic hypertension is unlikely to have been a significant confounding factor in the study groups.

TABLE 6 Intraobserver and interobserver coefficient of variation

	Intraobserver intracenter CV (%)	Interobserver intracenter CV (%)	Interobserver intercenter CV (%)
2D assessment			
Max-IVSd	3.3	3.7	4.2
Max-LVWd	5.2	5.9	5.9
LA max	3.2	3.7	5.0
LA/Ao	3.8	3.8	9.3
M-mode			
IVSd	4.2	10.7	11.4
LVWd	3.9	7.9	18.9

Abbreviations: 2D, 2-dimensional; IVSd, interventricular septum end-diastolic thickness; LA-max, maximum left atrial dimension; LA/Ao, ratio of the left atrial dimension to the aortic annulus dimension; LVWd, left ventricular free wall thickness at end-diastole; max-IVSd, maximum interventricular septum thickness at end-diastole; max LVWd, maximum left ventricular free wall thickness at end-diastole.

Prevalence of murmur in the Test group (100%) was higher than previously described because it was 1 of the key identifiers for the referred population in cats with sHCM.[43,53] In addition, 1 center actively recruited cats with cardiac murmurs. Heart murmurs were associated with dynamic LV outflow tract obstruction (DLVOTO), often termed hypertrophic obstructive cardiomyopathy (HOCM). Other studies have shown that cats with DLVOTO have a favorable prognosis compared to cats with clinical signs of HCM, as the disease is detected at an earlier stage.[61] Such earlier diagnosis might have contributed to the response to Test diet, as discussed above for cats without or with LA enlargement.

Some of the echocardiographic evaluations in this study have not been validated in cats but were used to enrich our understanding of the data. By assessing the number of areas ≥6 mm we aimed to detect diet effects that might occur in some areas without changing the overall maximum thickness. Additionally, changes in the sum of thicknesses in the evaluated regions would incorporate any diet effects on areas ≤6 mm. Interobserver variability could potentially have influenced the results, although the echocardiology measures used are common, and changes observed in max-IVSd and max-LVWd were larger than 5.9%, which was the highest interobserver CV for the primary endpoints. This CV is comparable to the overall interobserver CV for repeated measurements of echocardiography previously described in healthy cats.[62]

In summary, no differences between diet groups were significant for any variable at any timepoint. Cats fed a diet restricted in starch and supplemented with omega-3 fatty acids showed remodeling of the IVS and LVFW and decreased levels of cardiac marker cTnI, with changes being less evident in cats with LA enlargement. For the first time it was shown that diet could influence echocardiographic variables in cats with sHCM. This supports our hypothesis that manipulation of the levels of key dietary constituents might influence cardiomyocyte hypertrophy and thickness of the LV wall in cats with sHCM.

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CONFLICT OF INTEREST DECLARATION

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

The study was approved by the Royal Canin Ethics Committee, the University of Liverpool's Committee on Research Ethics (VREC335) and the University of Edinburgh's Committee on Research Ethics (VERC 93.16).

HUMAN ETHICS APPROVAL DECLARATION

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

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