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Associations among echocardiography, cardiac biomarkers, insulin metabolism, morphology, and inflammation in cats with asymptomatic hypertrophic cardiomyopathy

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Abstract

Background: Insulin, insulin-like growth factor-1 (IGF-1), and inflammation possibly are involved in cats with asymptomatic hypertrophic cardiomyopathy (aHCM).

Objectives: To evaluate echocardiography, morphology, cardiac and inflammatory markers, insulin and IGF-1 in cats with aHCM.

Animals: Fifty-one client-owned cats with aHCM.

Methods: Observational descriptive study. Variables (body weight [BW], body condition score [BCS], echocardiography, and serum concentrations of N-terminal pro-B-type natriuretic peptide [NT-proBNP], ultra-sensitive troponin-I [c-TnI], serum amyloid A [SAA], insulin, glucose and IGF-1) were evaluated for significant increases above echocardiography cutoff values and laboratory reference ranges, associations and effect of left atrial (LA) remodeling and generalized hypertrophy.

Results: Cats with aHCM had BCS ≥6/9 (P = .01) and insulin (P < .001), NT-proBNP (P = .001) and cTn-I (P < .001) above laboratory reference ranges. Associations were present between NT-proBNP and maximum end-diastolic interventricular septum thickness (IVSd; ρ = .32; P = .05), maximum end-diastolic left ventricular free wall thickness (LA/Aorta; ρ = .52; P = .001) and LA diameter (LA-max; ρ = .49; P = .003) and LA-max (ρ = .28; P = .05); and SAA and number of IVSd regions ≥6 mm thickness (ρ = .28; P = .05). Body weight and BCS were associated with IGF-1 (r = 0.44; P = .001), and insulin (ρ = .33; P = .02), glucose (ρ = .29; P = .04) and IGF-1 (ρ = .32; P = .02), respectively. Concentrations of

Abbreviations: aHCM, asymptomatic hypertrophic cardiomyopathy; Ao, aorta; APP, acute phase proteins; BCS, body condition score; BW, body weight; CHF, congestive heart failure; CRP, C-reactive protein; cTnI, cardiac troponin-I; HCM, hypertrophic cardiomyopathy; IGF-1, insulin-like growth factor – 1; IL-6, interleukin-6; IVRT, isovolumic relaxation time; IVS, interventricular septum; IVSd, end-diastolic interventricular septum thickness; LA, left atrium / atrial; LV, left ventricle / ventricular; LVFW, left ventricular free wall; LVWd, end-diastolic left ventricular free wall thickness; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PW-TDI, pulsed wave tissue Doppler imaging; RVFW, right ventricular free wall; RVOT/PA, right ventricular outflow tract / pulmonary artery; SAA, serum amyloid A; TNF-α, tumor necrosis factor-α.

Results were presented orally as an abstract at the 2018 American College of Veterinary Medicine Forum, Seattle, WA

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endothelial cells, and fibroblasts. Interleukin-6 has been shown to play a role in HCM. Although the assumed role of SAA is protective, it increases in response to inflammatory stimuli and can cause acute phase protein production.

### 1 | INTRODUCTION

In humans, insulin and insulin-like growth factor-1 (IGF-1) are critically involved in regulation of cardiomyocyte growth, by acting as an agonist on different signaling pathways including binding of insulin and IGF-1 to their receptors on the cardiomyocyte, stimulating myocardial protein synthesis and causing ventricular hypertrophy. A comparable mechanism possibly involving insulin resistance, the growth hormone-IGF-1 axis, or both may play a role in hypertrophic cardiomyopathy (HCM) in cats. Some, but not all, studies in cats with HCM have identified insulin resistance and increased growth hormone or IGF-1 concentrations. Maine Coon cats with HCM are skeletally larger and have higher IGF-1 concentrations than Maine Coon cats without HCM. Moreover, IGF-1 is associated with hypertrophy of the interventricular septum (IVS) and left ventricular free wall (LVFW). Cats with hypersomatotropism have a greater maximum LVFW, which decreases significantly after surgical hypophysectomy. Heart failure is associated with high LV filling pressures, hypoxia and tissue ischemia, and stimulating monocyte activation with subsequent release of cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Indeed, cats with congestive heart failure (CHF) caused by cardiomyopathies including hypertrophic cardiomyopathy have increased plasma concentrations of TNF-α. Interleukin-6 also can be produced by keratinocytes, endothelial cells, and fibroblasts. Interleukin-6 has been shown to cause myocyte hypertrophy, inhibition of cardiomyocyte apoptosis, alteration of protein synthesis, and stimulation of hepatocytes to produce acute phase proteins (APP) such as C-reactive protein (CRP) and serum amyloid A (SAA), facilitating release of APP into the blood. Progression of disease depends on the relative balance between pathological inflammatory pathways and physiological inflammation (ie, tissue reparative processes). Humans with HCM have evidence of systemic inflammation before developing CHF, but in cats it is unclear if, and at what stage, inflammation plays a role in HCM. Although the assumed role of SAA is protecting tissues from excessive damage caused by inflammatory mediators, IL-6 driven mechanisms will aggravate the ongoing process.

### 2 | MATERIALS AND METHODS

Ours was an observational descriptive study, evaluating age, BW, BCS, echocardiography, and serum concentrations of insulin, glucose, IGF-1, NT-proBNP, cardiac troponin-I (cTnl), and SAA in cats with aHCM. All owners signed an informed consent form before enrolling their cats in the study, and ethical approval was obtained from the Royal Canin Ethics Committee and University of Liverpool’s Committee on Research Ethics (VREC335) and University of Edinburgh Committee on Research Ethics (VREC 93.16). Adult cats 8 months of age and older were recruited from 4 different centers in the United Kingdom (University of Liverpool [UL], Anderson Moores Veterinary Specialists [AM], University of Edinburgh [UE] and North Downs Specialist Referrals [ND]). To be eligible, cats had to be diagnosed with HCM by echocardiography and be without clinical signs of heart disease (International Small Animal Cardiac Health Council stage 1b). Cats were excluded if they had important concurrent systemic disease based on history, physical examination, blood pressure, and laboratory testing (serum biochemistry profile and serum total thyroxine [T4] concentration). Cardiac medication was allowed but it had to have been administered for at least 8 weeks before inclusion. If cardiac medication was deemed clinically necessary after diagnosis of HCM, all examinations and analyses were postponed for a minimum of 8 weeks.

Physical examinations, BCS, blood pressure measurement, and echocardiography were performed by a board-certified cardiologist. Body condition score was assessed on a 9-point scale. Blood pressure was measured by Doppler technique using the mean of 3 systolic measurements, after recording at least 5 serial measurements, after acclimatization of the cat, and selecting 3 measurements that were within 10% of each other, to obtain the mean. Cats with systolic blood pressure >180 mmHg were considered hypertensive and excluded from the study. All cats had a full echocardiographic assessment, including left ventricular diastolic function and estimation of filling pressures.

We hypothesized that, in cats with aHCM, insulin, IGF-1 and inflammation possibly are involved in cardiac hypertrophy, and our objectives were to evaluate these factors in cats with aHCM.

**Conclusions and clinical importance:** Results suggest potential implications of insulin, IGF-1, and inflammation in cats with aHCM, but it remains to be confirmed whether these findings represent a physiological process or a part of the pathogenesis and development of disease.

**KEYWORDS**

cardiac hypertrophy, cat, hypertrophic obstructive cardiomyopathy, insulin/IGF-1 mediated growth
Echocardiography was performed on conscious cats without chemical restraint. Right parasternal 4- and 5-chamber long-axis views and short-axis views at different levels (papillary muscle, mitral valve, and aortic valve) were obtained. Two-dimensional (2D) measurements of the interventricular septum, the left ventricular cavity and left ventricular free wall at end of diastole and systole were made on the short-axis view at papillary muscle level, using blood-endocardial interface and myocardial-epicardial interface to guide caliper placement. The 2D interventricular septum and left ventricular free wall measurements were obtained from the right parasternal short- and long-axis (4- or 5-chamber) view of the left ventricle at end-diastole. The IVSd was measured in basal, mid, and apical regions, and LVWd was measured in basal and mid regions from right parasternal long axis 4- or 5-chamber views, whichever optimized any focal hypertrophy. The measurements were noted as thickness measured (max-) and the number of regions with ≥6 mm thickness (≥). Generalized hypertrophy was defined as thickness measured (max-) and the number of regions with ≥6 mm thickness (≥).

TABLE 1

| Variable               | Median   | Range     | Upper reference range | Number of cats with value above reference range | Mean value above reference range (P-value) |
|------------------------|----------|-----------|-----------------------|-----------------------------------------------|-------------------------------------------|
| Age (years)            | 4.42     | 0.92-17   | NA                    | NA                                            | NA                                        |
| BW (kg)                | 4.7      | 2.8-8.7   | NA                    | NA                                            | NA                                        |
| BCS                    | 5        | 3-9       | ≤5                    | 23                                            | .013*                                      |
| Max-IVSd (mm)          | 7        | 5.4-9.2   | ≤5.9                  | 48                                            | ≤.001*                                     |
| Max-LVWd (mm)          | 6.3      | 4.1-9.1   | ≤5.9                  | 33                                            | ≤.005*                                     |
| LA-max (mm)            | 16.4     | 11.4-23.4 | ≤15.9                 | 27                                            | .576                                       |
| LA/Ao                  | 1.29     | 0.97-2.33 | ≤1.49                 | 12                                            | 1                                         |
| Insulin (uU/mL)        | 22       | 3.9-113   | ≤11.4                 | 42                                            | ≤.005*                                     |
| Glucose (mmol/L)       | 5.9      | 3.8-11.2  | ≤7.8                  | 11                                            | 1                                         |
| IGF-1 (ng/mL)          | 405      | 81.2-786  | ≤665                  | 7                                             | 1                                         |
| NTproBNP (pmol/L)      | 179      | 26-1489   | ≤100                  | 26                                            | ≤.001*                                     |
| cTnI (ng/mL)           | 0.17     | 0.01-16.45| ≤0.06                 | 35                                            | ≤.001*                                     |
| SAA (mg/L)             | 0        | 0-21.8    | <3.9                  | 1                                             | 1                                         |

Abbreviations: BCS, body condition score; BW, body weight; cTnI, cardiac troponin-I; IGF-1, insulin-like growth factor-1; LA/Ao; LA-max, maximum diameter of left atrium; left atrium to aorta ratio; Max-IVSd, maximum thickness of interventricular septum in diastole; Max-LVWd, maximum thickness of left ventricular free wall in diastole; SAA, serum amyloid A.

*P value ≤.05.

Cats were fasted overnight before blood sampling. Blood was collected by cephalic or jugular venipuncture into serum tubes. Samples were divided into 3 aliquots and frozen at −20°C and shipped the following or same day to Idexx Bioscience, Germany. One aliquot was thawed on the day of arrival for biochemistry analysis (Beckman Coulter AU58000, Beckman Coulter, Brea CA), serum total T4 concentration (DRI Thyroxine, Microgenics, Fremont, California), c-TnI (ADVIA Centaur Tnl-Ultra Assay, Siemens Healthineers, Germany), glucose (Immulite 2000/Xpi, Siemens Healthineers, Germany), IGF-1 (CLIA, Siemens, Immulite 2000, Idexx, Germany) and SAA (LAA, Elken Chemical, Beckman Coulter AU58000, Idexx, Germany). A second aliquot was stored frozen at −80°C, followed by shipment on dry ice to the United Kingdom for analysis of insulin (IRMA, Beckman Coulter, Nationwide, UK). The remaining aliquot was thawed on the day of arrival or frozen at −80°C and thawed within 2 days for analysis of NTproBNP (Feline Cardiopet NT- proBNP Immunoassay, Idexx, Germany).

All continuous variables are described by their median and range (minimum-maximum). To test if there was a significant increase beyond the reference range of echocardiographic, BCS, and blood measurements, a 1 sample 1-sided t test and a Wilcoxon 1-sided signed rank test were used, for normally and non-normally distributed data, respectively. Because in our investigation the study group was from a different population than the population that generated the reference ranges, tests for independent groups were applied when performing statistical analysis. To test for significant differences (increase or decrease) in BCS, echocardiographic and blood variables between groups positive or negative for LA remodeling, general hypertrophy or receiving cardiac medication, a 2-sample 1-sided t test was used when both samples were normally distributed.
distributed or a Wilcoxon 1-sided ranked test was used otherwise. To explore for possible associations between 2 different measurements, the Pearson correlation for normally (and also homogeneously) distributed continuous variables, and Spearman correlation for non-normally distributed continuous or continuous-ordinal variables, were used. To investigate if a relationship existed between 2 categorical variables, a Chi-square test was performed because the data were unpaired. Because there were many comparisons within the same hypothesis (eg, in the block of LA remodeling, there were 17 comparisons), it was decided to keep the test procedure exploratory so as not to miss any results of interest. Consequently, a significance level of .05 was applied for all tests. All statistical analyses were performed using SAS software version 9.4 (2014).

### RESULTS

#### 3.1 Study population

Fifty-one cats were recruited from the 4 centers between November 2015 and June 2017. Numbers of cats from the centers differed, with 25 cats from UL, 19 cats from AM, 4 cats from UE, and 3 cats from ND. Seventeen cats were female and 34 cats were male. There were 36 domestic short- and long-hairs, 4 British shorthairs, and 11 cats of other breeds (3 Selkirk Rex, 1 Maine Coon, 1 Exotic Shorthair, 1 Persian, 1 Persian cross, 1 Bengal, 1 Siamese, 2 Ragdoll cross). Forty-eight cats had a murmur on cardiac auscultation. Nine cats had been receiving cardiac medication for a minimum of 8 weeks at the time of evaluation (atenolol [n = 6], or a combination of atenolol and clopidogrel [n = 2] or telmisartan [n = 1]).

#### 3.2 Descriptive results

Table 1 shows the descriptive results for age, BW, BCS, echocardiography, and blood variables, and significant increases above echocardiographic cutoff values and laboratory reference range where applicable. Median systolic blood pressure was 131 mmHg (range, 120-142 mmHg).

### Table 2: Number of cats with hypertrophic regions in IVSd and LVWd

| Number of regions ≥6 mm | LVWd | 0 | 1 | 2 |
|-------------------------|------|---|---|---|
| IVSd                    | NA   | 0 | 3 |
| 1                       | 7    | 5 | 3 |
| 2                       | 4    | 4 | 4 |
| 3                       | 7    | 3 | 11 |

Abbreviations: IVSd, interventricular septum in diastole; LVWd, left ventricular free wall in diastole.

### Table 3: Descriptive results of cats with LA remodeling, generalized hypertrophy, and receiving cardiac medication

| Variable                  | LA remodeling (n = 27) | Generalized hypertrophy (n = 11) | Receiving cardiac medication (n = 9) |
|---------------------------|------------------------|----------------------------------|-------------------------------------|
| Age (years)               | 4.65 0.92-12.8 .594    | 6.1 0.92-13.92 .143              | 7.5 1.7-13.7 .229                   |
| BW (kg)                   | 4.7 2.8-6.8 .242       | 5.6 2.8-8.7 .077                 | 4.7 3.5-6.8 .544                    |
| BCS                       | 6 4-7 0.054            | 6 4-9 .239                       | 6 4-7 .500                         |
| Max-IVSd (mm)             | 7 5.5-9.2 .146         | 8.0 6.0-9.2 .003* <.001 b         | 7.6 6-9.2 .066                      |
| Max-LVWd (mm)             | 6.7 4.9-9.1 .016 b     | 7.3 6-9.1 <.001 b                | 6.6 5.1-8.0 .409                    |
| LA-max (mm)               | 17.1 11.4-23.4 <.001 b | 16.8 12.6-20 .067               | 16.7 13.0-23.4 .086                 |
| LA/Ao                     | 1.34 0.97-2.33 .014 b  | 1.28 1.0-2.0 .673                | 1.3 1.2-2.3 .030 p                  |
| Insulin (uU/mL)           | 21.5 6.3-76 .630       | 16.7 6-53 .859                   | 23 8.9-33 .530                      |
| Glucose (mmol/L)          | 5.9 3.8-9.5 .519       | 5.9 4.2-9.5 .709                 | 6.1 5.1-9.5 .264                    |
| IGF-1 (ng/mL)             | 461 81.2-786 .132      | 482 81.2-730 .432                | 405 328-786 .412                    |
| NTproBNP (pmol/L)         | 350 27-1489 .016 b     | 290 42-1489 .500                 | 487 97.0-964 .082                   |
| cTnl (ng/mL)              | .32 0.02-16.5 .009 a   | .33 0.02-16.5 .087               | 0.28 0.03-1.74 .078                 |
| SAA (mg/L)                | 0 20-21.8 .103        | 0.1 0-21.8 .018 a                | 0 0-21.8 .188                       |

Abbreviations: BCS, body condition score; BW, body weight; cTnl, cardiac troponin-I; IGF-1, insulin-like growth factor-1; LA/Ao, left atrium to aorta ratio; LA-max, maximum diameter of left atrium; Max-IVSd, maximum thickness of interventricular septum in diastole; Max-LVWd, maximum thickness of left ventricular free wall in diastole; SAA, serum amyloid A.

*Decreased values compared to cats without LA remodeling/general hypertrophy/medication P decrease value = 1 – P increase value.

bP value < .05.
Both NT-proBNP and cTnI had missing data with results above and below the detection limit, although sensitivity analysis showed comparable conclusions with and without imputations.

On echocardiography, cats had focal or multifocal hypertrophy but the largest number of cats had generalized hypertrophy with hypertrophy in all regions of IVSd and LVWd (Table 2). Of the 51 cats, 28 cats had LA remodeling, 11 cats had generalized hypertrophy, and 7 cats had both LA remodeling and generalized hypertrophy.

Of the 28 cats showing LA remodeling, 17 cats had either LA max ≥16 mm (n = 16) or LA/Ao ≥1.5 (n = 1), and 11 cats had both LA max ≥16 mm and LA/Ao ≥1.5. In cats with LA max ≥16 mm, LA/Ao ≥1.5 or both, LA max ranged from 11.4 to 23.4 mm, with a median of 17 mm, and LA/Ao ratio ranged from 0.97 to 2.33 with a median of 1.3. Cats with LA remodeling had significantly higher MAX-LVWd on echocardiography and significantly higher blood concentrations of NT-proBNP and cTnI compared to cats without LA remodeling (Table 3).

Cats with generalized hypertrophy had significantly higher SUM-LVWd and LA-max on echocardiography, and significantly higher blood concentrations of SAA, compared to cats without general hypertrophy (Table 3).

Cats receiving cardiac medication for a minimum of 8 weeks only had significantly higher LA/Ao (Table 3).

### 3.3 Associations

Correlation analysis showed significant associations between several of the evaluated variables. Results are shown grouped according to echocardiography, cardiac biomarkers, insulin, glucose, IGF-1, and SAA, as shown in Table 4.

#### TABLE 4  Significant associations between variables from different parts of the hypothesized mechanism

| Echocardiography          | Cardiac biomarkers | Insulin, glucose, IGF-1 | SAA |
|---------------------------|-------------------|-------------------------|-----|
| Age                       |                   |                         |     |
| BW n-LVWd                |                   |                         |     |
| BCS                       |                   |                         |     |
| IVSd                      |                   |                         |     |
| LA                        |                   |                         |     |
| LA remodeling             |                   |                         |     |
| LA max                    |                   |                         |     |
| LA/Ao                     |                   |                         |     |
| Glucose                   |                   |                         |     |
| Insulin                   |                   |                         |     |
| IGF-1                     |                   |                         |     |
| SAA                       |                   |                         |     |
| NT-proBNP                 |                   |                         |     |
| c-TnI                     |                   |                         |     |

Abbreviations: BCS, body condition score; BW, body weight; cTnI, cardiac troponin-I; IGF-1, insulin-like growth factor-1; LA/Ao, left atrium to aorta ratio; LA max, maximum diameter of left atrium; Max-IVSd, maximum thickness of interventricular septum in diastole; Max-LVWd, maximum thickness of left ventricular free wall in diastole; n-IVSd, number of regions in interventricular septum in diastole ≥6 mm; n-LVWd, number of regions of left ventricular free wall in diastole ≥6 mm; SAA, serum amyloid A.

#### DISCUSSION

We hypothesized that insulin, IGF-1, and inflammation possibly may be involved in cardiac hypertrophy in cats with aHCM. We evaluated these variables as well as age, BW, BCS, echocardiography, and cardiac markers NT-proBNP and cTnI.

The blood concentration of insulin was significantly above laboratory reference range in cats with aHCM. Forty-two of the 51 cats (82%) were hyperinsulinemic with insulin concentrations up to 113 uU/mL (Table 1). Blood glucose concentration ranged from 3.8 to 11.2 mmol/L, with 30 of the 42 cats with insulin concentration above laboratory reference range having normal fasting blood glucose concentrations. Cats were fasted overnight before blood sampling and clinically relevant hyperglycemia (as in diabetes) was excluded, but it is possible that cats experienced mild hyperglycemia associated with stress. Biological variability and insulin concentrations above the reference range in clinically healthy cats have been described previously, but using a different assay for determination of serum insulin concentrations. Previous studies have shown that insulin concentrations were comparable in healthy cats with and without LVH, and between Maine Coon cats with and without HCM. This might be a specific finding in the Maine Coon breed and in our study 1 Maine Coon cat did not have a blood insulin concentration above reference range. High insulin concentrations are associated with LVH in clinical studies of humans. Through Akt signaling pathways stimulating protein synthesis and inhibiting protein breakdown in cardiomyocytes, insulin plays an important role in cardiac hypertrophy in humans. Diabetic cardiomyopathy has been described in humans, with hyperinsulinemia proposed as 1 of the pathogenic mechanisms. Existence of a comparable cardiomyopathy related to hyperinsulinemia in cats remains controversial. Although diastolic dysfunction and increased risk of
Cats were subdivided based on the presence or absence of LA remodeling and generalized hypertrophy. Cats with LA remodeling or generalized hypertrophy did not have significantly increased blood concentrations of insulin, IGF-1, or glucose, but cats with LA remodeling had significantly increased NTproBNP and cTnI concentrations. Increased concentration of NTproBNP previously have been reported to be associated with LA remodeling, and NTproBNP is higher in severe HCM. Serum concentration of cTnI also is higher in severe HCM and is described to be higher in cats >10 years of age, possibly reflecting a subclinical age-related decrease in cardiac function. However, this age is much higher than the median age of the cats with LA remodeling in our study.

Serum amyloid A concentrations were not significantly increased above laboratory reference range in the overall population, but were higher in cats with generalized hypertrophy compared to cats with focal or multifocal hypertrophy, and were associated with the number of hypertrophied regions in the interventricular septum. Because no age-matched control group was included, it is unclear whether this association is only present in cats with aHCM, or whether it is related to the pathophysiology of the disease. Serum amyloid A is a major APP in cats, and is the most rapidly responsive APP to an inflammatory trigger. It is unclear whether the higher SAA concentrations in cats with generalized hypertrophy represent a physiologic (ie, protective) or pathologic mechanism. The release of IL-6 from activated monocytes causes myocyte hypertrophy, as well as release of APP, such as SAA in the blood to protect tissues from excessive damage. Median SAA concentrations in cats with generalized hypertrophy were within laboratory reference range, hence only local inflammation might be present, with limited monocyte activation. Inflammation has been associated with CHF in different cardiac diseases and species, including cats with HCM. In addition, histological evidence suggests that cats with preclinical HCM have mild inflammatory cell infiltrates in the myocardium. Local inflammation could explain the higher SAA concentrations in cats with generalized hypertrophy compared to cats with focal or multifocal hypertrophy and the correlation of SAA with the number of hypertrophied regions, but it is unclear why SAA concentration is associated with number of hypertrophied regions in the interventricular septum and not in the left ventricular free wall. Interestingly, SAA has been described to be higher in cats with diabetes mellitus compared to healthy cats, but whether any relationship exists with insulin or IGF-1-mediated growth remains unclear. Other inflammatory markers such as serum TNF-α and IL-6 were not evaluated in our study. We did not perform immunohistochemistry for inflammatory infiltrates in the myocardium, because cats were living and obvious ethical reasons excluded endomyocardial biopsies, which could have contributed to the identification of local inflammation in cardiac tissue.

Nine cats received atenolol either alone or combined with telmisartan or clodipogrel. Treating asymptomatic cats with HCM appears rational, but to date there is no proof of a treatment benefit on morbidity, quality of life, or survival. Atenolol treatment previously has been reported to have no effect on NTproBNP or cTnI, suggesting that atenolol has no effect on cardiac stretch or cardiomyocyte injury. In our study, cats receiving cardiac medication...
Hypertrophic cardiomyopathy in cats is a naturally occurring model with genotypic and phenotypic similarities to HCM in humans, although the disease is more severe and progresses more quickly in cats. In humans, circulating concentrations of IGF-1 are related to the extent of myocardial injury, and regional expression of IGF-1 is increased in the myocardium of patients with HCM. It is unclear whether in cats exogenous modulation of the insulin-IGF-1 axis might be beneficial in the management of cardiovascular disease with increased myocardial mass as in humans with HCM. Benefits of omega-3 fatty acids on the production of inflammatory mediators known to be increased in heart failure remain to be evaluated in cats.

Ninety-four percent of cats in our study had murmurs, which is higher than previously described. The population in our study came from referral centers and 1 center actively searched for cats with cardiac murmurs, which possibly increased the number of cats with cardiac murmur in our study. Sixteen percent of cats were receiving cardiac medications, which is lower than previously described.

The evaluation of ventricular wall thickness is done routinely by noting the maximum measured value at any region of the interventricular septum or LV wall. The additional evaluation of noting the number of areas (3 in the interventricular septum and 2 in the left ventricular wall) is not a validated method. Nonetheless, this technique was used to diagnose generalized hypertrophy with a thickness ≥6 mm measured in all 5 areas, and gives interesting insight into the severity of cardiac hypertrophy in asymptomatic cats and its association with cardiac biomarkers, SAA and BW.

A major limitation of our study was the absence of an appropriate control group of age-matched healthy cats without cardiac disease, preferably from the same geographic location and referral centers. Because of this limitation, our results can only be regarded as exploratory. It is unclear whether the described associations also are present in healthy cats, or whether they represent an important finding in the pathogenesis of aHCM. Interpretation of the results was limited to comparing them to widely established cutoff values for echocardiographic measures and laboratory reference ranges for blood analysis. Laboratory reference ranges are generated from a healthy cat population and validated by the laboratory performing the analysis.

Asymptomatic HCM is very common in cats, with a prevalence approaching 15% in apparently healthy cats. Interventions that could alter progression of aHCM into CHF, arterial thromboembolism or death are still to be defined. Although results from our study suggest potential implications of insulin, IGF-1 and inflammation in the pathophysiology of aHCM in cats, their precise role as factors in the development of disease and potential therapeutic targets to slow progression remains to be confirmed.

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CONFLICT OF INTEREST DECLARATION
Ingrid van Hoek is an employee of Royal Canin SAS. Royal Canin SAS did not influence the study.

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, University of Liverpool’s Committee on Research Ethics (VREC 335) and University of Edinburgh Committee on Research Ethics (VREC 93.16).

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

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