AN ABSTRACT OF THE THESIS OF

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Title: Inotropic and Chronotropic Effects of Sotalol in Healthy Dogs

Abstract approved:

Katherine F. Scollan

Sotalol is a class III anti-arrhythmic drug with class II properties that is commonly used in the treatment of ventricular tachyarrhythmias in dogs. The anti-arrhythmic effects of sotalol are mediated by an increase in action potential duration and prolongation of atrial and ventricular repolarization via antagonism of the delayed rectifier potassium current. These effects have been demonstrated to be independent of sotalol's beta-blocking properties. However, beta blockade may result in reduced myocardial contractility and also contribute to slowing of the sinus rate. A cardiodepressant effect is of clinical importance when sotalol is used in patients with existing heart disease, yet the inotropic properties of sotalol are not well characterized. The aim of this study was to investigate the inotropic and chronotropic effects of sotalol on healthy, awake dogs.

Ten healthy dogs were recruited from the faculty, staff, and students of the Oregon State University College of Veterinary Medicine. Eligible dogs were between one and six years of age and weighed over 20 kilograms (kg). Dogs were considered healthy based on history, physical exam, oscillometric blood pressure measurement, transthoracic echocardiography, and a 10-lead electrocardiogram. Each dog was also evaluated with a 24-hour baseline Holter monitor. Sotalol at a dose of 1-2 mg/kg was

then administered for 12-16 days, followed by a second evaluation including the same diagnostics tests. Physical exam parameters, heart rate (HR) and arrhythmias on Holter recording, blood pressure, standard echocardiographic measurements, three-dimensional (3D) left ventricular (LV) volumes, and 3D strain were measured at each evaluation. Measurements from before and after treatment were compared with paired t-tests for normally distributed data and Wilcoxon signed rank tests for non-normally distributed data. 3D data were available for 7/10 dogs. No correction was made for multiple comparisons.

Ten dogs were included in the study, with a mean age of 3.4 years (range, 1.1-6.4 years) and mean weight of 26.14 kg (range, 21-35.8 kg). Each dog received 40 mg sotalol orally every 12 hours, resulting in a mean dose of 1.56 ± 0.23 mg/kg. HR on exam was significantly ($p = 0.036$) lower post-treatment (81 ± 23 bpm) than pre-treatment (101 ± 26 bpm). Maximum HR on Holter monitor was also significantly ($p = 0.002$) lower post-treatment (195 ± 14 bpm) than pre-treatment (215 ± 13 bpm). Fractional shortening (FS) using two-dimensional (2D) and M-mode (MM) measurements was significantly reduced post-treatment (2D 27.4 ± 4; MM 24.9 ± 5.7%) compared to pre-treatment (2D 30.7%, IQR 28.7-33.8; MM 32.5 ± 2.6%) with $p = 0.01$ and $p = 0.004$ for 2D and M-mode measurements, respectively. Similarly, ejection fraction (EF) via Simpson's method of disks (SMOD) was significantly ($p = 0.002$) lower post-treatment (48 ± 6.8%) than pre-treatment (53.8 ± 4.4%). Post-treatment there was also a 12.3% (95% CI: 6.4-18.2%) increase in LV end-systolic dimension on 2D and a 12.6% (95% CI: 3.7-21.5%) increase on M-mode measurements ($p = 0.001$, $p = 0.01$, respectively). There was no significant difference between pre- and post-treatment in the 3D left ventricular volumes, nor in global longitudinal or circumferential strain, twist, or torsion.

The results of this study indicate that sotalol has a negative inotropic and chronotropic effect in healthy dogs. Standard 2D and M-mode echocardiographic measurements used to assess systolic function showed a small but statistically significant decrease after sotalol treatment, with a mean reduction in EF (SMOD) of 5.8%. The lack of significance based on 3D imaging may reflect the variability in measurement and the small sample size. The effect of sotalol should be assessed in dogs with heart disease to further elucidate the clinical significance of the reduction in systolic function, and the implications of this reduction in patients at risk for heart failure.

Inotropic and Chronotropic Effects of Sotalol in Healthy Dogs

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Dean of the College of Veterinary Medicine

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Julia Rice Treseder, Author

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CONTRIBUTION OF AUTHORS

Dr. Treseder solicited study participants and organized data collection. Drs. Treseder, Scollan, and LeBlanc designed the study. Dr. Treseder performed the majority of data collection with the assistance of Drs. Scollan and LeBlanc. Dr. Treseder performed measurements and Drs. Treseder, Scollan, and LeBlanc collectively participated in data interpretation. Dr. Treseder authored the thesis, which was edited by Drs. Scollan and LeBlanc.

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INTRODUCTION

Sotalol is an anti-arrhythmic agent with class II and class III properties used in human and veterinary medicine for control of ventricular and supraventricular arrhythmias. Interest in sotalol's particular anti-arrhythmic properties arose in the 1970s when its ability to prolong the action potential duration was discovered during studies of various beta-blockers.[1-3] Sotalol and amiodarone are the prototypical class III anti-arrhythmics that spurred the investigation into newer, "pure", class III compounds.[3] Although considered promising, use of class III drugs for ventricular arrhythmias in humans has been limited after d-sotalol (the dextro isomer of sotalol which exhibits primarily class III properties) was shown to increase mortality in patients with systolic dysfunction after myocardial infarction.[4] Sotalol is still used for suppression of ventricular arrhythmias,[5-7] but the use of ventricular anti-arrhythmic drugs in human medicine has shifted with the advent of implantable cardiac defibrillators (ICDs), which are superior to medical therapy in the prevention of sudden death.[8] However, sotalol remains in widespread use for conversion and maintenance of sinus rhythm in people with atrial fibrillation.[9] In contrast, sotalol remains a mainstay of therapy for ventricular arrhythmias in veterinary medicine, where ICDs are rarely used, and is also used on occasion for supraventricular arrhythmias.[10-14] However, there are few prospective studies on its efficacy and safety in veterinary patients.

Sotalol's anti-adrenergic properties present a concern for exacerbation of underlying systolic dysfunction. The label recommendation for human use includes caution when initiated in patients with left ventricular dysfunction or congestive heart failure (CHF) based on a one-year incidence of new or worsened CHF of 3% in patients without a prior history and 10% in those with a prior history of CHF.[15] Despite their negative inotropic effects, beta-blockers as a class are part of standard therapy in

patients with heart failure with reduced ejection fraction; the guidelines acknowledge a risk for worsening heart failure, but recommend re-instituting therapy in the long term based on a reduced risk for major clinical events.[16]

In veterinary patients with systolic dysfunction with or without the presence of CHF, the safety of sotalol is based on anecdote and expert opinion rather than empirical evidence. Indeed, among veterinary cardiologists, there is a lack of consensus in general on the therapeutic effect of beta-blockade in patients with heart disease. Beta-blockers are not routinely used for the treatment of the most common acquired canine heart diseases, namely degenerative valve disease and dilated cardiomyopathy. A few studies have been conducted that have shown no benefit nor apparent harm on neurohormonal or echocardiographic progression of disease. However, many of these were small studies with a relatively short follow-up period, or were limited by a retrospective study design and lack of a control group.[17-20] For dogs with degenerative valve disease, consensus guidelines include a minority of experts advocating for the use of beta-blockers in the preclinical and clinical periods, with slow up-titration after resolution of heart failure signs.[21] Thus, the potential negative side effects associated with beta-blockade from sotalol administration in acquired structural heart disease, especially those cases affected with systolic dysfunction or in CHF, remain controversial in veterinary medicine.

Although beta-blockers are used uncommonly in acquired canine heart disease, they are used often in congenital diseases that lead to pressure overload such as pulmonic stenosis and subaortic stenosis. The theoretical benefit relates to negative inotropic and chronotropic effects which reduce myocardial oxygen demand,[22-24] as well as the anti-arrhythmic potential of beta-blockers to reduce the risk for sudden death.[25] The effect of beta-blockers on the action potential varies depending on the underlying

disease and the relative potency of the drug's action on different adrenergic receptors. Direct effects on ionic currents include a reduction in the pacemaker funny current (I_f) and inward calcium currents (I_{Ca}) and varied effects on repolarizing potassium currents.[25] Indirect effects may include beta-receptor downregulation and a decrease in myocardial oxygen consumption. Some or all of these may contribute to an anti-arrhythmic effect in a given clinical scenario.[25] However, there are few studies on long-term outcomes of these diseases and even fewer which evaluate the efficacy of medical therapy; those that have been published have failed to show a survival benefit with beta-blockers.[26-29] Medical therapy is of particular importance in severe subaortic stenosis, which is accompanied by a grave prognosis and a lack of effective surgical or interventional options.[26,27,30-33] Sotalol, by virtue of its prolongation of the action potential duration, has the potential to confer benefit beyond traditional (and unproven) beta-blocker therapy with a more potent anti-arrhythmic effect.

There is widespread use of sotalol in veterinary medicine, primarily as an anti-arrhythmic agent for ventricular arrhythmias. However, there is little understanding of its effect on ventricular function in a clinical setting, which has implications for patients affected with a wide variety of heart diseases. The goal of this study was to characterize the inotropic and chronotropic effects of sotalol in a population of healthy dogs.

Electrophysiologic effects of sotalol

Sotalol, available as a racemic mixture of *D*- and *L*-isomers, has anti-adrenergic effects and prolongs the action potential, thereby exhibiting class II and class III anti-arrhythmic properties, respectively.[1,15,34] The cardiomyocyte action potential consists of four phases determined by ionic currents. Depolarization (phase 0) is followed by brief repolarization (phase 1) and a plateau (phase 2). When outward potassium currents

activated during the plateau phase exceed inward sodium and calcium currents, rapid repolarization (phase 3) begins.[35] The delayed rectifier potassium current (I_K) has the largest effect on repolarization and consists of a rapidly activated (I_{Kr}) and a slowly activated (I_{Ks}) component.[36] The refractory period, during which the cell is unresponsive to stimuli, occurs when the cell is depolarized at less negative membrane potentials during phases 1, 2, and early phase 3.[35] Sotalol's concentration-dependent increase in action potential duration (APD) is mediated by prolongation of atrial and ventricular repolarization and thus the effective refractory period, which has been demonstrated in both dogs and humans and is independent of its beta-blocking action (Figure 1).[3,37-41]

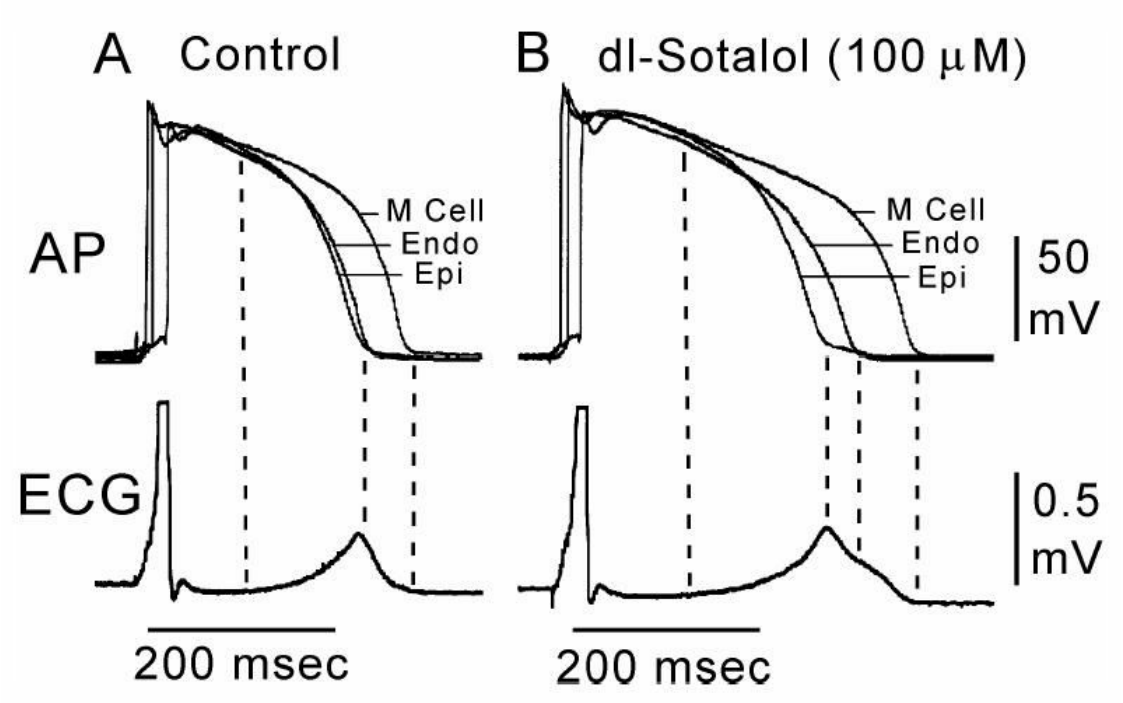

Figure 1: Action potentials and transmural ECG recorded from canine left ventricular wedge preparation, control (A) and arterially perfused with sotalol (B). From Fenichel, et al.[42,43]

Sotalol acts via blockade of I_K, essentially entirely via the rapid component with negligible effect on I_{Ks}; there may also be a small reduction in the inward rectifier

potassium current, $I_{K1,}$ and transient outward current, I_{to}, both of which also contribute to repolarization.[40,44,45] Sotalol exhibits reverse use dependence, so that its prolongation of the action potential is greater at slower heart rates.[37,46] This is perhaps mediated by a greater contribution of I_{Ks} to the repolarizing current with shorter diastolic intervals, so that the inhibitory effect of sotalol on I_{Kr} plays less of a role.[40] Sotalol's prolongation of APD appears to be further exaggerated in CHF based on an experimental study in dogs with tachycardia-induced cardiomyopathy.[47] This may be related to downregulation of myocardial potassium channels in heart failure, which would limit recruitment of additional repolarizing currents in response to sotalol, or to an enhanced sympatholytic effect in the context of the higher catecholamine levels present in heart failure.[47,48]

Increasing the APD prolongs refractoriness to reduce the probability of fibrillation and widens the cycle length of sustained tachycardias to prevent deterioration into fibrillation,[3] and sotalol has been demonstrated to have anti-fibrillatory potential.[40,49] The increase in wavelength also prevents establishment of a stable reentrant circuit, so that a reentrant arrhythmia is more likely to be terminated.[3,50]

The class II action of sotalol is nonselective and almost entirely attributable to the *L*-isomer.[37] Sotalol's potency with regards to beta-blockade varies between studies, but oral formulations are generally considered to have ¼ to ½ the potency of propranolol, which is a nonselective beta-adrenergic antagonist.[37,38] Beta-blockade has multiple effects on the pacemaker action potential, including inhibition of spontaneous depolarization (phase 4) via inhibition of the funny current, I_f, the likely mechanism by which sotalol reduces the sinoatrial rate.[25,51,52] Sotalol also causes a decrease in conduction velocity and prolongation of refractoriness in the atrioventricular node, similar to other beta-blockers, suggesting this is at least in part via inhibition of catecholaminergic effects on calcium and potassium channels.[25,38,52,53]

Hemodynamic effects of sotalol

The increase in intracellular calcium concentration triggered by the action potential determines the maximal force developed by the myocardium. Sotalol would be expected to exhibit a negative inotropic effect associated with a reduction in calcium availability, either from direct blockade of adrenergic receptors or by inhibition of the force-frequency response. The force-frequency relationship describes the increase in the force of ventricular contraction that occurs with an increase in heart rate.[54] This is due to an increase in calcium influx at a high rate of stimulation of the cardiac myocyte, which may be mitigated at the slower heart rates seen with sotalol. However, it has also been suggested that the longer APD mediated by sotalol allows a greater influx of calcium, leading to an increase in contractility.[38,40] The Frank-Starling response might also contribute to improved contractile function because of a longer diastolic filling time resulting in a higher preload.[55]

Contractility is considered an inherent feature of the myocardium independent of preload and afterload, but is difficult to measure because of the interconnectedness of contractile function and loading conditions.[56,57] It is estimated experimentally in isolated muscle tissue by directly evaluating the force-velocity and force-length relationships.[57] In the intact heart, assessment of contractile function often relies on measurement of pressure-volume loops or extrapolation from force-velocity data (e.g. to derive the rate of early pressure development or the maximum velocity under “zero” loading conditions).[57,58] Ejection phase indices are used to estimate the work of the ventricle or the volume it ejects, but are more likely to be influenced by preload and afterload.[57]

Experiments on isolated papillary muscle from cats have shown that sotalol has a neutral or positive effect of on contractility,[1,59-62] and one study on isolated canine

ventricular trabecular muscle revealed both an increase in APD and force development.[63] A more recent study in isolated normal human left ventricular myocardium, however, showed a negative inotropic effect which was primarily attributed to beta-blockade.[64]

While *in vitro* animal studies suggested an increase in contractility, *in vivo* experiments performed under controlled conditions in various animal species have failed to demonstrate a consistent positive inotropic effect. Experiments in rats have shown a reduction in contractility in normal and post-ischemic models, included under controlled loading conditions.[65,66] Studies in anesthetized dogs have been variable, with some studies demonstrating no or ambiguous effects on contractility,[67,68] and others documenting both negative inotropic and negative chronotropic effects of sotalol.[69,70] In one study on paced, anesthetized dogs, sotalol preserved contractility in conjunction with prolongation of the APD, including preventing a reduction in contractility at longer pacing cycle lengths (i.e. blunting the downslope of the force-frequency relationship).[46]

Studies performed in humans with heart disease have demonstrated negative inotropic effects with intravenous infusions of sotalol. Several early studies reported a reduction in heart rate, evidence of reduced myocardial oxygen consumption, and some also showed a reduction in parameters of systolic function. These included one study in which patients' heart rate was controlled via artificial pacemaker and a decrease in left ventricular (LV) dP/dt (the rate of the rise in left ventricular systolic pressure[71]) was found upon infusion of sotalol.[67,72,73] A more recent study showed an approximately 11% reduction in LV dP/dt in patients with stable symptoms undergoing cardiac catheterization for chest pain after receiving intravenous sotalol, which was similar to the reduction with metoprolol and verapamil.[74] The same study evaluated the effects of different cardiac cycle lengths and determined that sotalol exhibited intermediate

attenuation of the force-frequency response, with metoprolol preserving the force-frequency response and verapamil virtually ablating it.[74]

Echocardiographic evaluation of ventricular function

Echocardiography is the routine method of assessing ventricular function in veterinary medicine because it is accessible, affordable, and can be performed without the need for sedation or anesthesia. However, parameters of ventricular function derived from echocardiographic imaging are load-dependent, and as such reflect the interaction of preload, afterload, and contractility.[71,75] *In vitro* experiments allow for assessment of contractility in terms of force, length, velocity and time during isotonic contraction of isolated muscle preparations, although clearly not a reflection of clinical conditions.[57] Relatively load-independent measures, such as pressure-volume loops and dP/dt, can be calculated in the catheterization laboratory with increasing ease and accuracy given the advances in catheter technology.[71,76] Advanced imaging techniques, including nuclear medicine, computed tomography (CT), and magnetic resonance imaging have also expanded the ability to assess ventricular function in recent decades.[77,78] However, because echocardiography is available, portable, and provides real-time dynamic imaging of the heart *in vivo*, it remains the most common noninvasive modality used in human medicine as well as veterinary medicine for quantification of heart chamber size and function.[79]

Echocardiographic indices of left ventricular systolic function include linear, two-dimensional (2D), and Doppler techniques used in standard echocardiographic examination. As discussed above, these should not be seen as indicators of myocardial contractility alone. For example, an increase in preload will cause a rise in ejection phase indices based on the Frank-Starling mechanism, and an increase in afterload will

reduce ejection fraction; however, the interaction is complex in that an increase in preload will generally also increase left ventricular dimensions and systolic pressure, thereby also increasing afterload.[71] Therefore, echocardiographic measures of systolic function must be interpreted in light of the complex interactions of preload, afterload, and contractility, in addition to consideration of the inherent limitations of ultrasound.

Linear measurements such as fractional shortening (FS) are limited in that they interrogate along a single line, and therefore do not reflect regional variation in shape and function.[75] E-point to septal separation (EPSS) is an indirect marker of left ventricular function that correlates with the volume of transmitral flow and left ventricular stroke volume, but this becomes inaccurate in the presence of mitral regurgitation.[75] Despite their limitations, linear measurements based on M-mode (MM) echocardiography remain in common use in veterinary medicine based on their ease of acquisition, and their relevance in common canine diseases in which assessment of global left ventricular size and function has clinical implications.[80-82]

Two-dimensional area measurements, which also reflect function only at the level being interrogated, are uncommonly used for assessment of left ventricular function. Currently, ventricular volumes are often calculated using Simpson's method of discs (SMOD) from a 2D image (four-chamber or two-chamber view), this formula having supplanted earlier methods of volume estimation that relied more heavily on geometric assumptions.[75] From these end-diastolic and end-systolic volume estimates, stroke volume (SV) and ejection fraction (EF) are derived. The American Society of Echocardiography recommends the biplane SMOD using the left apical four- and two-chamber views.[79] In dogs, it is common to use the right parasternal four-chamber view and/or the left apical four-chamber view because of the difficulty in obtaining left apical two-chamber views,[83] and these single plane methods have generally shown good

agreement with each other.[84-86] End-systolic volume indices have also been suggested as a more preload-independent measure of systolic function than ejection fraction.[87,88]

Doppler-derived indices of systolic function include the time velocity integral of the left ventricular outflow tract to measure stroke volume, which is subject to measurement error especially in determining the area of the outflow tract, and the myocardial performance index (MPI) or Tei index, which includes components of both systolic and diastolic function.[75] Measurement of dP/dt uses the early curve of the mitral regurgitation spectral profile to assess isovolumic contraction, analogous to dP/dt measured with a micromanometer catheter; this measure is relatively load-independent but depends on the presence of mitral regurgitation.[75]

Tissue Doppler imaging (TDI) is a relatively recent advancement in echocardiography used in the quantitative assessment of systolic function. TDI modes include pulsed-wave, color M-mode, and 2D color TDI, which can be used to evaluate myocardial velocity.[89,90] Indices often used to assess systolic function include annular systolic velocities and deformation analysis (e.g. strain and strain rate).[75] Strain rate, the change in velocity between two points, is measured using TDI, from which strain, the normalized change in length between two points, is calculated by integrating over time.[75] Strain can be calculated in orthogonal planes to assess longitudinal, circumferential and radial contraction. Normal contraction involves negative longitudinal systolic strain (shortening of the myocardial segment) and then biphasic diastolic strain.[75] TDI is useful in evaluation of global and regional myocardial dysfunction and ventricular synchrony, and indeed may be a more sensitive technique than traditional echocardiography, but is limited by angle dependency and the propensity for artifacts.[89-91]

Two-dimensional speckle tracking echocardiography (STE) tracks the location of a discrete myocardial segment based on the location of speckle patterns, natural

acoustic markers created by interference between the ultrasound beam and the myocardium.[75,90] It is a more recent tool that has the advantage of being independent of cardiac translational motion and less reliant on insonation angle compared to Doppler-based techniques, although it still depends on image quality and therefore tracks best along the ultrasound beam where there is better resolution.[79,90] Two-dimensional STE calculates strain, from which strain rate is derived. Twist and torsion, measures of apical rotation relative to the heart base (the "wringing" motion of the ventricle), can also be assessed with TDI and STE techniques.[75,90] The most common strain-based measure of global left ventricular systolic function is global longitudinal strain (GLS), usually assessed with STE, and in human medicine it is considered to provide incremental prognostic data over ejection fraction.[79] However, because measurements vary based on measurement position and software, universal normal values are not yet defined in the human literature; a general guideline is that a peak GLS of -20% is expected in a healthy person.[79] Initial studies in veterinary medicine have shown that 2D STE has the potential to be a repeatable, reproducible method of evaluating systolic function, although its clinical relevance is not well established.[92-96] Normal longitudinal strain measurements in healthy dogs appear similar to or slightly lower than those for humans (one study found a GLS of -16.61 ± 2.21 in anesthetized, healthy dogs),[95] and measures of radial and circumferential strain also appear similar.[92,97,98] However, torsion is more variable and absolute values differ from those in humans.[93,99,100]

Historically, the left heart has been the major focus of both human and veterinary echocardiographic assessment, however, recently there has been increasing recognition of the importance of the right heart. Guidelines in human medicine recommend comprehensive assessment of the right heart including quantitative measures of right ventricular systolic function.[79,101] Indices of right ventricular systolic function include

tricuspid annular plane systolic excursion (TAPSE) obtained from M-mode images, fractional area change (FAC) on 2D images, right ventricular index of myocardial performance (RIMP) and lateral tricuspid annular systolic velocity using Doppler and TDI, respectively, as well as strain, strain rate, and ejection fraction.[79] These indices have also been the subject of recent investigation to establish canine reference ranges in the veterinary literature,[102-106] as well as to evaluate their clinical importance.[107,108] Because of the complex anatomy of the right ventricle, volumetric measurements such as EF are best performed using three-dimensional echocardiography (3DE) which obviates the need for geometrical shape assumptions required to calculate volumes from two-dimensional images.[101]

Three-dimensional echocardiography

The emergence of 3DE along with the advancements in its acquisition and interpretation have led to the increasing use of 3DE in human cardiology.[109] Currently, full-volume data sets of the left or right ventricle are acquired using ECG-gated multi-beat acquisitions which stitch together several pyramidal subvolumes.[110] This is known as real-time 3DE (RT3DE), which represents a significant technological advancement over previous methods of off-line reconstruction to create 3D volumetric data.[111] One vendor offers the capability of single-beat full volume acquisition in real-time, although at the expense of spatial resolution.[112] A systematic review and meta-analysis of 3DE in humans showed that left ventricular volumes were consistently underestimated (compared to cardiac magnetic resonance (CMR) imaging as the gold standard) with substantial variability; however, 3DE showed better accuracy and precision in measuring left ventricular volumes and better precision in measuring left ventricular ejection fraction compared with standard 2D echocardiography.[113] For evaluation of right ventricular (RV)

systolic function, 3DE with semiautomatic border detection has proven reliable.[79] However, similar to the LV, right ventricular volumes and EF are also underestimated compared to CMR.[114] 3DE is recommended for assessment of left ventricular volume and function in experienced laboratories when there is good image quality, and it is similarly encouraged for evaluation of right ventricular systolic function when feasible.[79]

In veterinary medicine, the use of 3DE is limited primarily to research institutions and is rarely used in clinical practice. Initial verification of left ventricular volumetric data using 3DE has showed underestimation of volumes compared to CMR and multidetector row CT (MDCT) imaging, similar to humans.[83,115,116] Two of the three studies on 3DE showed good correlation of full-volume data sets with MDCT or CMR,[83,115] with the third showing weak to moderate correlation with CMR.[116] Each of these studies verified the feasibility of 3DE for evaluation of left ventricular size and function, albeit in anesthetized dogs. Validation of right ventricular volumetric data using 3DE has also been performed in veterinary medicine with comparison to a volumetric gold standard.[106]

A newer application of 3DE is in deformation analysis using STE. The limitations of 2D STE include non-simultaneous data acquisition, allowing short-term variations in function to impact measurements, and the 'out-of-plane' phenomenon in which the region of interest moves out of the ultrasound beam during the cardiac cycle.[117] 3D STE avoids these limitations and also allows acquisition of all strain parameters with a single full-volume data set.[112] It also permits the measurement of area strain, a measure of deformation of the LV endocardial surface area which integrates longitudinal and circumferential strain.[118] However, it does rely on a good acoustic window and good temporal resolution.[112] Initial validation studies have shown good correlation of 3D STE with sonomicrometry, although less so for radial strain, and good correlation between strain components and ejection fraction. Vendor dependency hinders reproducibility, yet

reproducibility is generally still considered at least acceptable.[112,117] Most strain components measured in 2D correlate with those in 3D, however, reference values are different due to different software algorithms and the inclusion of twisting cardiac motion in 3D STE. Although 3D STE has not yet been fully validated for clinical use, there is increasing interest in 3D STE for the evaluation of subclinical cardiac disease and prognostication, including diseases affecting the right heart.[112,117,119-122] In veterinary medicine, this emerging technique has not yet been studied.

Study hypothesis and aims

The aim of this study was to characterize sotalol's inotropic and chronotropic effects in healthy dogs and provide a basis for future research in dogs affected with heart disease. We hypothesized that treatment with oral sotalol would have negative chronotropic effects and echocardiographically detectable negative inotropic effects on ventricular function in healthy, awake dogs.

MATERIALS AND METHODS

Enrollment

Ten healthy, adult (1-6 years old), large-breed (>20 kg) dogs were prospectively recruited from the veterinary community at the Oregon State University College of Veterinary Medicine. Dogs were eligible for participation provided that the owner had given informed consent. Dogs with a history of a murmur, arrhythmia, or clinical signs of cardiopulmonary disease were excluded. Dogs were also excluded if they were currently being administered any medications, with the exception of monthly preventative drugs for ecto- and endoparasites.

Dogs were evaluated with a complete physical examination, oscillometric blood pressure (BP), 10-lead electrocardiogram (ECG) and standard echocardiogram. Dogs were excluded from the study if there was evidence of clinically significant systemic or cardiovascular disease upon initial assessment. Exclusion criteria therefore included the detection of a murmur or abnormal pulmonary sounds on thoracic auscultation, auscultation or documentation of a pathologic arrhythmia on ECG, and echocardiographic evidence of hemodynamically significant congenital or acquired structural heart disease. Dogs were eligible for inclusion if there was evidence of trivial degenerative valve disease, i.e. if there were characteristic lesions of the mitral valve and/or trivial mitral regurgitation on echocardiogram in the absence of a murmur, cardiac chamber enlargement, or evidence of systolic dysfunction.

This study was approved by the Institutional Animal Care and Use Committee at Oregon State University.

Protocol

Upon inclusion in the study, baseline evaluation consisted of physical examination, BP, ECG, standard two-dimensional, M-mode, and Doppler echocardiography, three-dimensional echocardiography and a 24-hour Holter monitor, as described below. Sotalol was then prescribed at 1-2 mg/kg orally twice daily. Post-treatment evaluation occurred 12-16 days after initiation of sotalol administration, and included the same diagnostic tests. Each dog then underwent a short tapering protocol consisting of a reduction in dose frequency to once daily administration for 3 days, after which sotalol was discontinued.

Electrocardiography

A 10-lead ECG (MAC 5500, GE Healthcare, Freiburg, Germany) was performed in unsedated dogs restrained in right lateral recumbency. ECG was performed according to standard technique and electrical activity was recorded for 15-30 seconds.[123] The underlying heart rhythm and presence of pathologic arrhythmias was noted.

Blood pressure measurement

Oscillometric blood pressure measurement (CARDELL Veterinary Monitor 9401, Midmark, Tampa, FL) was performed by an experienced technician prior to other diagnostic testing to minimize stress from handling and restraint. Dog were unsedated and gently restrained in right lateral recumbency. Each dog was fitted with a blood pressure cuff estimated to be 30-40% of the limb's circumference, and the cuff was applied to the mid-metatarsal region of the left hindlimb. Five consecutive measurements of systolic, diastolic, and mean arterial pressure were recorded; measurements associated with the highest and lowest mean arterial pressure, or obvious outliers of

systolic or diastolic blood pressure, were discarded. The mean of the remaining three measurements of systolic, diastolic and mean arterial pressures were recorded.

Echocardiography

Dogs were unsedated and restrained in right and left lateral recumbency. All dogs underwent M-mode, 2D, Doppler, and RT3DE imaging with simultaneous ECG recording (iE33, Philips Medical Systems, Andover, MA). Two-dimensional imaging was obtained with an 8-3 MHz phased array or X5-1 MHz matrix transducer, and all three-dimensional imaging was obtained with an X5-1 MHz matrix transducer. All 2D echocardiograms were performed by the same echocardiographer (JT); RT3DE images were obtained by at least two echocardiographers for each study (JT and NL or KS).

Standard echocardiography

Measurements were performed for three consecutive cardiac cycles except where image quality was considered inadequate, in which case fewer or non-consecutive cardiac cycles were measured. The mean value was recorded.

M-mode imaging: M-mode images of the left ventricle were obtained from the right parasternal (RPS) short axis view at the level of the papillary muscles. Left ventricular free wall thickness at end-diastole (LPWd) and end-systole (LPWs), interventricular septal wall thickness at end-diastole (IVSd) and end-systole (IVSs), LV internal diameter at end diastole (LVIDd), and LV internal diameter at end systole (LVIDs) were measured with wall thicknesses measured from leading edge to leading edge (Figure 2). Fractional shortening was calculated according to the formula (LVIDd-LVIDs)/LVIDd x 100. M-mode images of the LV at the level of the mitral valve were also obtained from the RPS short axis view, and EPSS was measured. M-mode images from the left apical four-chamber view optimized for the right heart were obtained with the

cursor parallel to the RV free wall. These images were used to measure TAPSE as previously described.[102,124]

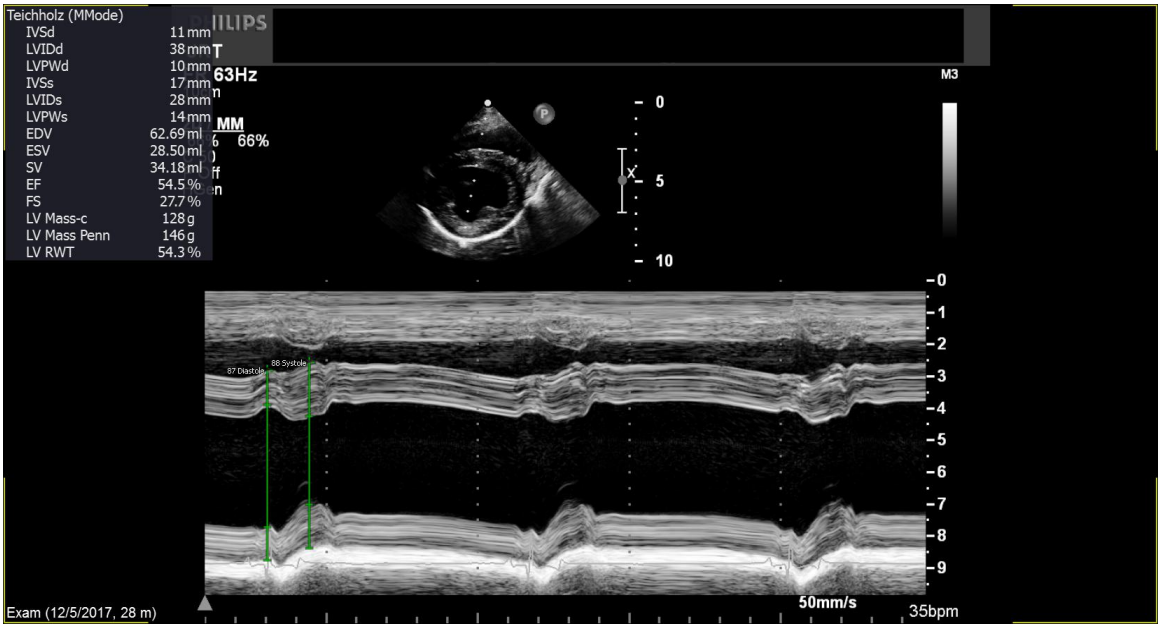

Figure 2: M-mode of the left ventricle at the level of the papillary muscles with linear measurements of the left ventricle performed at end-diastole and end-systole.

2D imaging: Standard 2D imaging planes were obtained in each dog.[125] LPWd, LPWs, IVSs, IVSd, LVIDd and LVIDs were measured from the RPS short-axis view at the level of the papillary muscles with wall thicknesses measured from leading edge to trailing edge. Fractional shortening was calculated according to the same formula used to calculate M-mode FS. The ratio of the left atrial to aortic root diameter (LA/Ao) was measured from the RPS short-axis basilar view as previously described.[126] Left ventricular end-systolic and end-diastolic volumes were measured using monoplane SMOD from the RPS long axis four-chamber view (Figure 3).[127] The endocardial border was traced to include the papillary muscles within the measured chamber volume. End-diastolic volume was measured when the mitral valve was closed and the left ventricular

volume was at its largest, selected from frames at the onset of the QRS. The frame used to measure end-systolic volume was the last frame before mitral valve opening. Left ventricular end-diastolic (EDV) and end-systolic (ESV) volumes were recorded and used to calculate EF according to the formula (EDV-ESV)/EDV x 100. Left apical four-chamber views optimized for the right heart were obtained as previously described (Figure 4) and used to measure right ventricular fractional area change (RV FAC).[102] Right ventricular end-diastolic area (RVAd) was measured with the tricuspid valve closed and the right ventricular area at its largest, and right ventricular end-systolic area (RVAs) was measured at the last frame before tricuspid valve opening with the right ventricular area at its smallest. Right ventricular FAC was calculated according to the formula (RVAd-RVAs)/RVAd x 100.

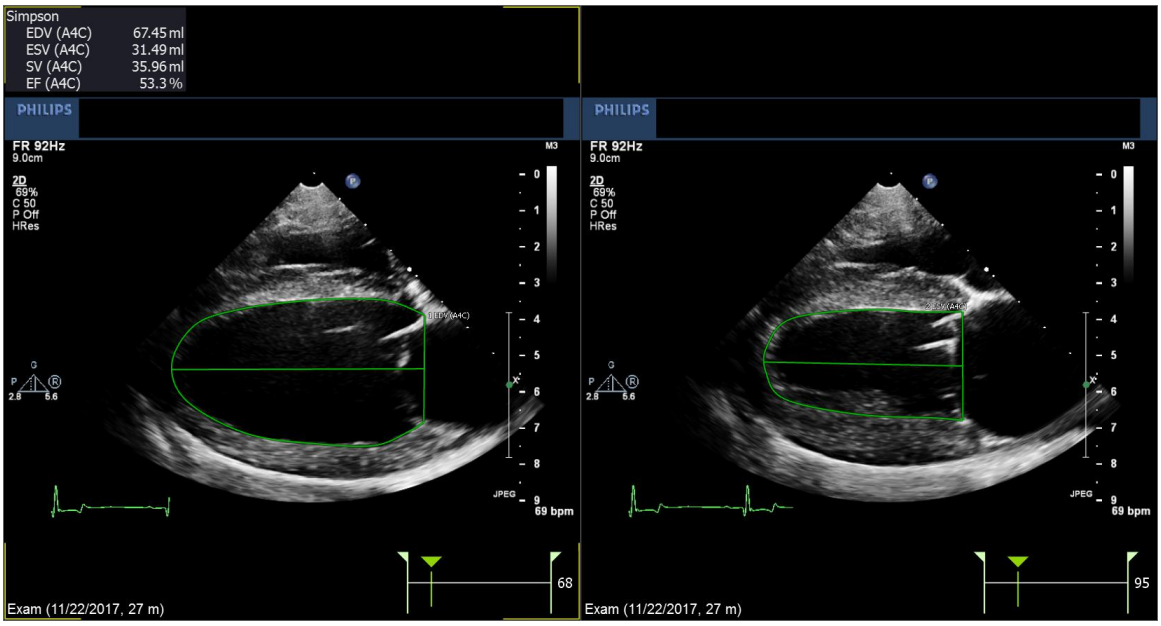

Figure 3: 2D right parasternal long axis four-chamber view with measurement of left ventricular volume using Simpson's method of discs.

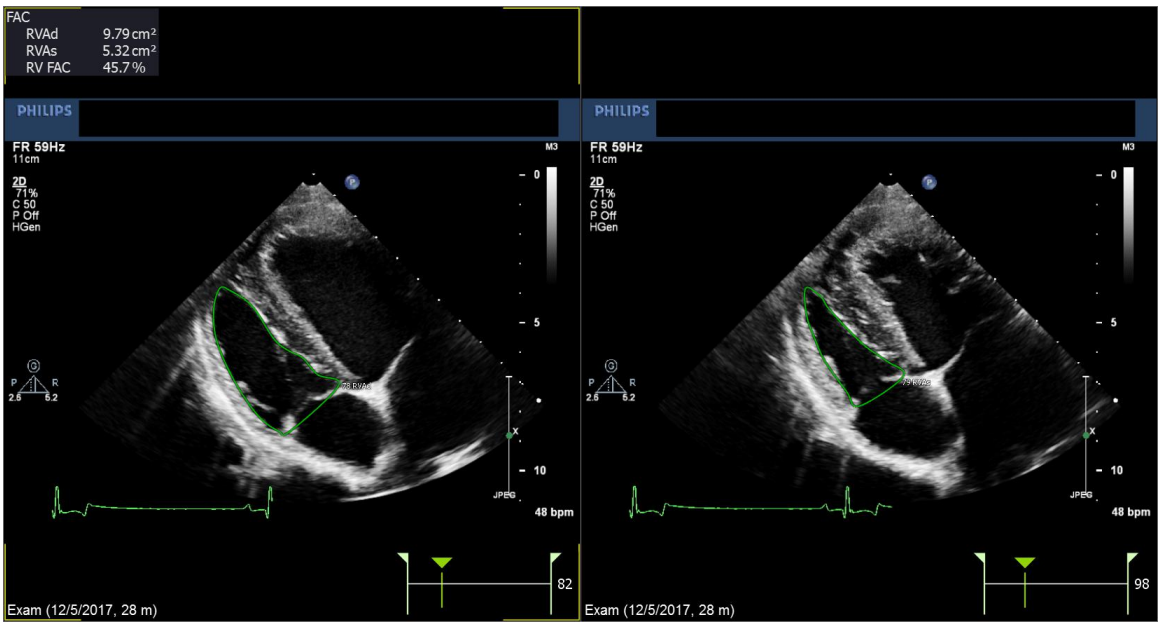

Figure 4: 2D left apical four-chamber view optimized for the right heart with measurement of right ventricular fractional area change.

Spectral and color flow Doppler imaging: Pulsed wave Doppler was performed at the level of the pulmonic valve from the RPS short axis-basilar view and the peak pulmonic outflow velocity was measured. The peak aortic outflow velocity was measured from continuous wave Doppler images obtained from the subcostal or left apical five-chamber view. The same view was used in an individual dog for baseline and post-treatment measurements. Pulsed wave Doppler was also performed to assess mitral inflow with interrogation at the level of the open mitral valve leaflet tips from the left apical four-chamber view. The E wave was identified as the first positive wave in early diastole (following the T wave on the ECG) and the A wave was identified as a positive wave after the P wave on the ECG. The maximal E and A wave velocities were measured to calculate the peak E wave to peak A wave ratio (E/A). Valvular insufficiency and the presence of turbulent flow were subjectively assessed with color flow Doppler.

Tissue Doppler imaging: Pulsed wave tissue Doppler imaging was performed from the left apical four-chamber view at the lateral mitral annulus, septal mitral annulus (Figure 5), and lateral tricuspid annulus, with the latter performed from a left apical view optimized for the right heart.[102,128,129] The E' and A' waves were identified as negative waves in early and late diastole, respectively, using the ECG for reference as described for mitral inflow measurements. The S' wave was identified as the positive wave immediately after the QRS complex. The peak E wave to peak E' wave ratio (E/E') was calculated using the maximal E' wave velocity on TDI of the lateral mitral annulus. Peak S' was recorded for the lateral and septal mitral annulus and the lateral tricuspid annulus.

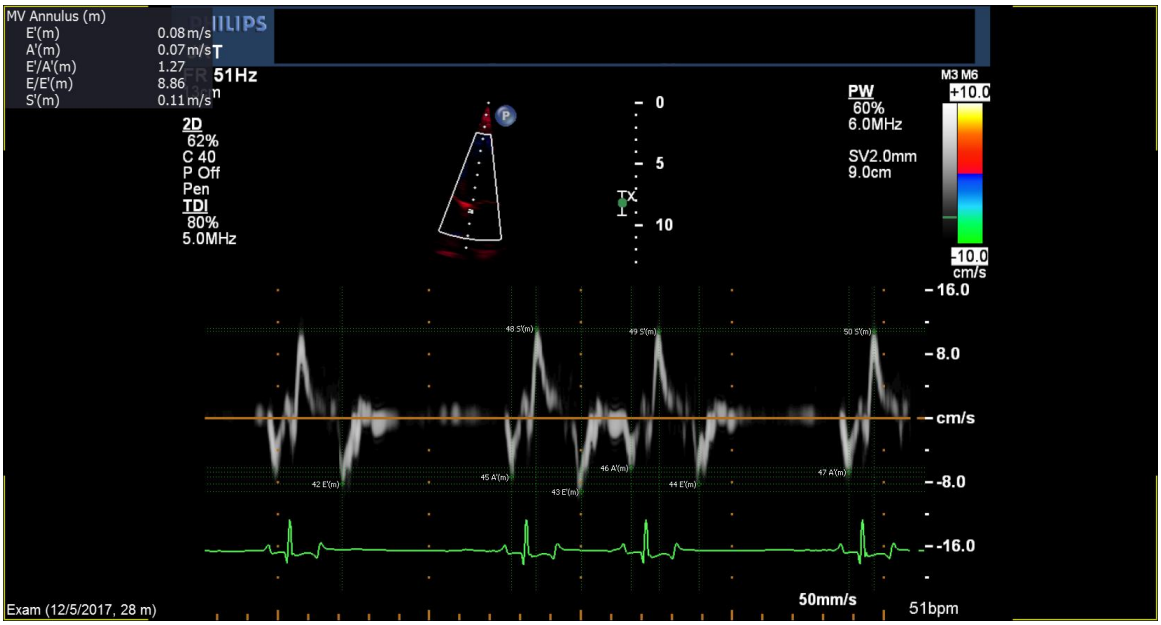

Figure 5: Pulsed-wave tissue Doppler imaging of the septal mitral annulus from the left apical four-chamber view with measurement of the E', A', and S' waves.

3D echocardiography

Image acquisition: Real-time 3DE images of the left ventricle were obtained from the RPS long axis four-chamber view and from the left apical four-chamber view. RT3DE images of the right ventricle were obtained from the left apical four-chamber view optimized for the right heart. Full volume, ECG-gated 3D images were obtained with pyramidal volumes expanded to encompass the chamber of interest. Full volume 3D data sets were acquired from subvolumes of four consecutive cardiac cycles. Each recorded loop contained five full-volume data sets. Data were acquired after the R wave was correctly identified on simultaneous ECG, with an attempt to minimize artifact by acquiring images during periods of regular rhythm and quiet respiration. The frame rate was between 15 and 30 Hertz.

Image interpretation: Data were stored digitally and analyzed off-line using commercially available software (4D LV-ANALYSIS, TOMTEC Imaging Systems GMBH, Munich, Germany). End-diastolic and end-systolic frames were automatically identified by the software. Reference points (apex, mitral annulus, and aortic valve for the LV; LV apex, mitral annulus, tricuspid valve, RV apex, and RV septal hinge points for the RV) were manually identified for appropriate chamber orientation on multiple views (four cross-sectional planes for the LV, Figure 6; six planes for the RV, Figure 7), and contour tracing was performed with semi-automatic border detection. For the LV, three long axis cross-sectional planes were used to manually adjust the endocardial border tracing with an additional short axis plane within the volume data to confirm and correct endocardial border tracing (Figure 8). For the RV, short axis cross-sectional planes were used for manual border adjustment with a long axis plane used to adjust the short axis view and confirm border tracing (Figure 9). Automatically calculated volume and strain measurements of interest were recorded (Figures 10 and 11): end-diastolic volume

(EDV), end-systolic volume (ESV), stroke volume (SV), ejection fraction (EF), global longitudinal strain (GLS), global circumferential strain (GLS), twist, and torsion. Measurements were performed on a single data set (i.e. one complete heartbeat) within a series of five consecutive cardiac cycles.

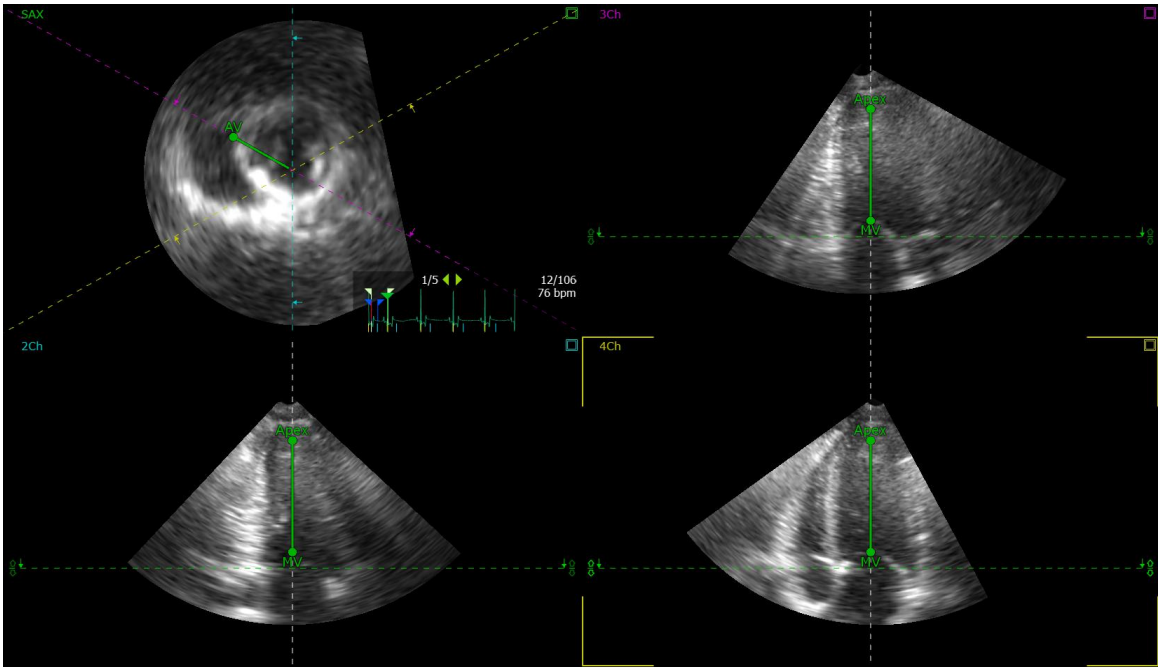

Figure 6: Analysis of RT3DE imaging of left ventricle from left apical four-chamber view: Identification of reference points. Note poor resolution for border detection.

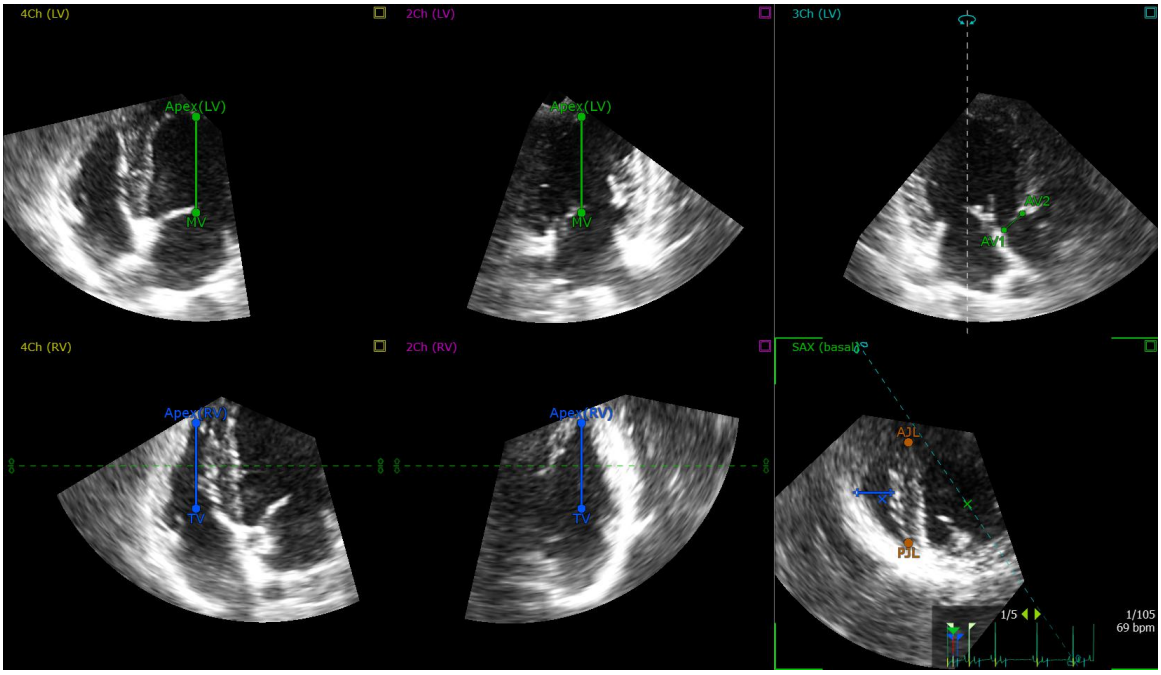

Figure 7: Analysis of RT3DE imaging of right ventricle: Identification of reference points.

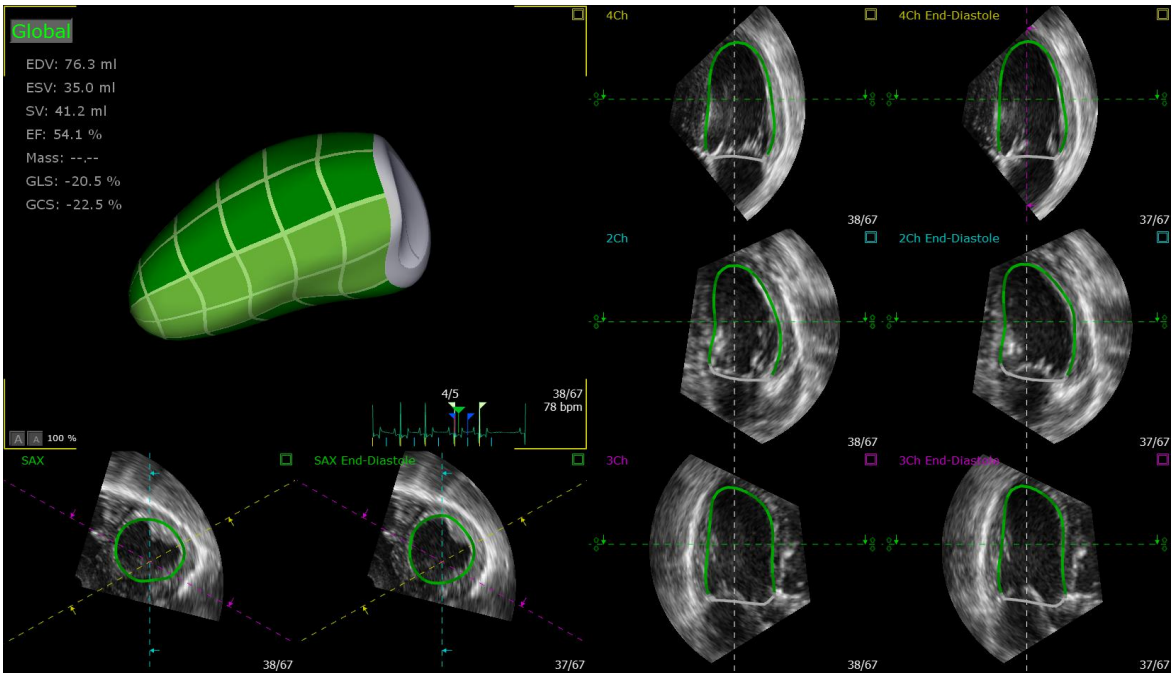

Figure 8: Analysis of RT3DE imaging of left ventricle: Tracking revision following semi-automatic border detection.

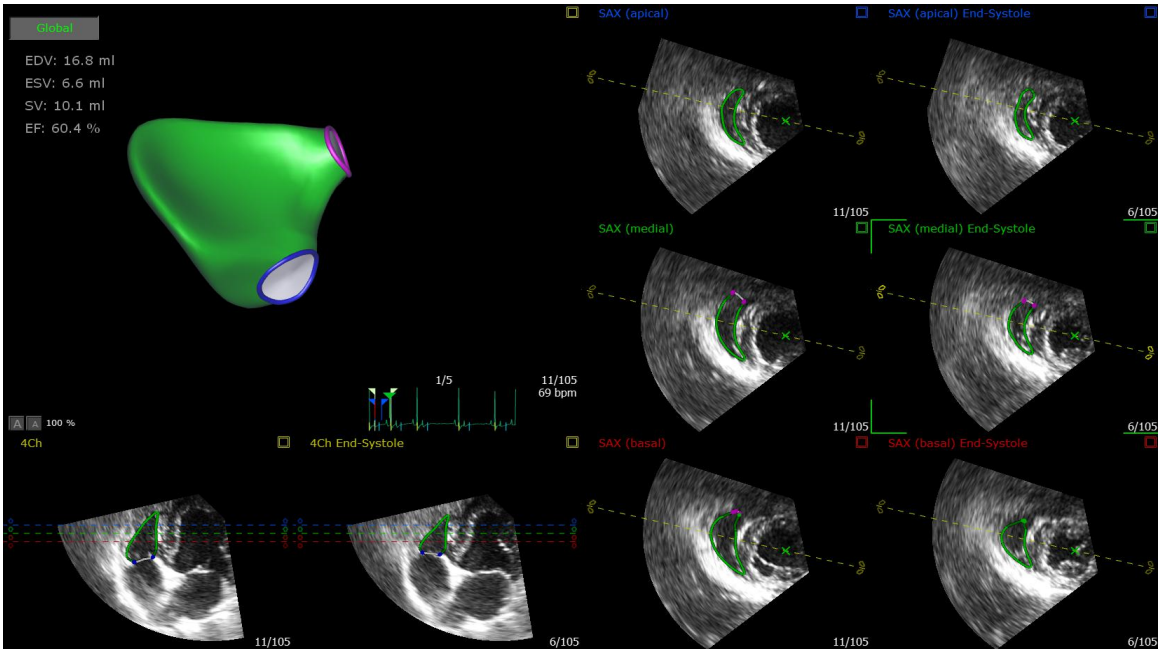

Figure 9: Analysis of RT3DE imaging of right ventricle: Tracking revision following semi-automatic border detection.

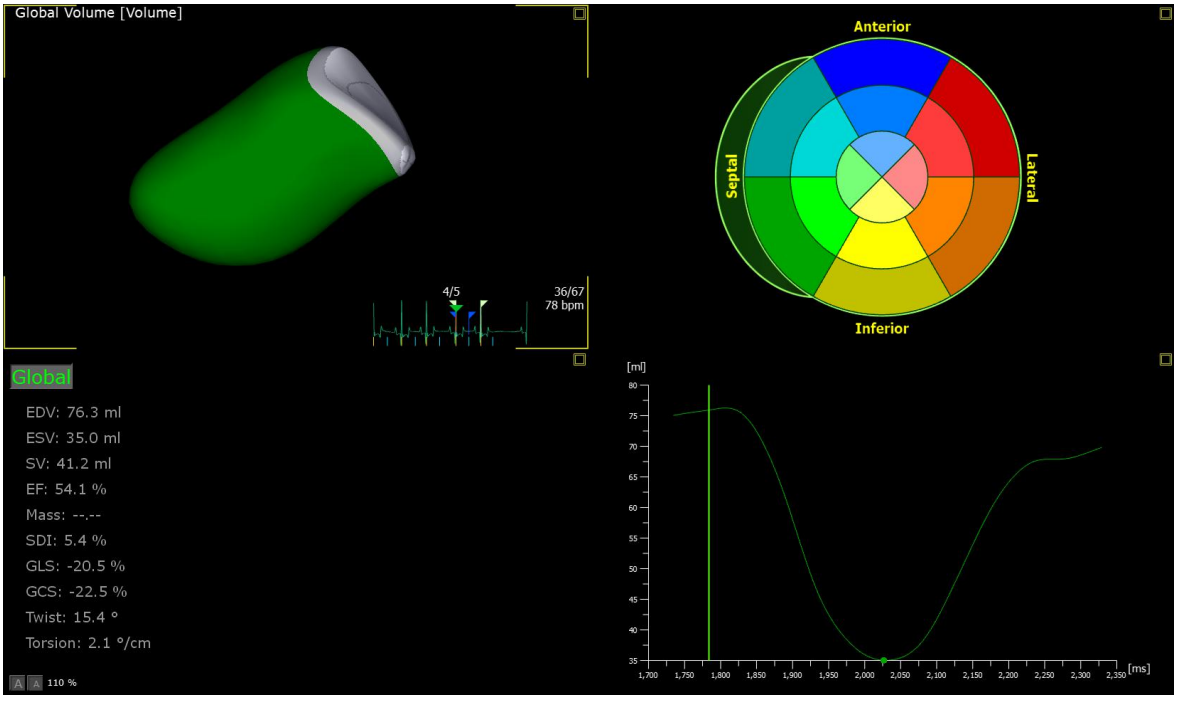

Figure 10: Analysis of RT3DE imaging of left ventricle: Global volume.

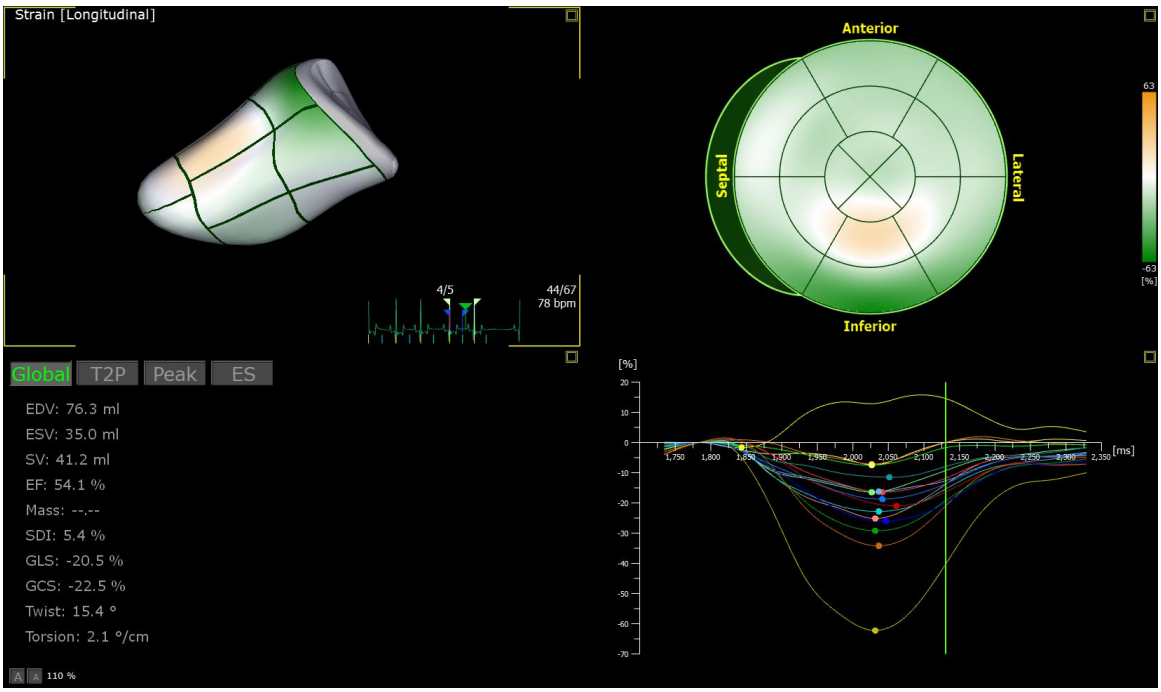

Figure 11: Analysis of RT3DE imaging of left ventricle: Longitudinal strain.

Holter monitoring

A 24-hour ambulatory electrocardiogram (AECG) using a digital, 3-channel transthoracic system (Trillium 5900, Forest Medical, LLC, East Syracuse, NY) was performed. Each dog was sent home after AECG placement to allow recording in the dog's normal environment. Owners were encouraged to maintain the dog's normal activity level and follow a typical routine. The AECG was removed after 24 hours and AECG digital files were analyzed by a veterinary cardiology resident (JT) under the supervision of a veterinary cardiologist (NL and KS) with an AECG analysis system (Trillium Platinum Holter analysis software, Forest Medical, LLC, East Syracuse, NY). The total number of ventricular premature complexes (VPCs) per 24-hour period, and maximum, mean, and minimum heart rate were tabulated. The number of sinus pauses longer than 3 seconds was also recorded for each Holter. The occurrence of other pathologic arrhythmias was noted, if present.

Statistical analysis

Statistical analyses were performed with commercial analysis software (Prism 7, GraphPad Software, San Diego, CA). Normal distribution of continuous data was assessed with the D'Agostino & Pearson test. Baseline and post-treatment data were compared with paired t-tests for normally distributed data and Wilcoxon signed rank tests for non-normally distributed data. Volumetric measurements performed in 2D were compared to 3D data with Wilcoxon signed rank tests. A two-sided alpha of $p < 0.05$ was considered significant. No correction was made for multiple comparisons. Normal data are expressed as mean ± standard deviation (SD) or mean (SD) and non-normal data are expressed as median values with interquartile range (IQR). The difference between means is expressed as mean (95% confidence interval).

RESULTS

Study population

Ten dogs were recruited between September 2016 and November 2017, all of whom were included in the study. Baseline data are presented in Table 1. The mean age was 3.4 ± 1.9 years (range, 1.1-6.4 years) and mean weight was 26.1 ± 4.3 kg (range, 21.0-35.8 kg). The mean heart rate was 101 ± 26 bpm, systolic blood pressure was 127 ± 11mmHg, diastolic blood pressure was 69 ± 14mmHg, and mean blood pressure was 91 ± 10mmHg. The mean sotalol dose administered was 1.56 ± 0.23 mg/kg. All dogs were in sinus rhythm on baseline ECG.

Heart rate

The post-treatment heart rate on physical exam was 20 bpm (95% CI: 2-38 bpm) lower than the baseline heart rate ($p = 0.036$).

Blood pressure

There was no significant difference between baseline and post-treatment blood pressure.

Standard echocardiography

All dogs had normal chamber dimensions, wall thickness, and systolic function on baseline echocardiogram, although 3/10 dogs (30%) had trace mitral regurgitation (MR) on at least one of the two echocardiograms performed. Trace to mild aortic insufficiency (AI) was noted in 2/10 dogs (20%); both of these dogs also had trace MR. One dog (with trace MR and mild AI) who was 6 years of age was diagnosed with mild degenerative valve disease. Trace to mild pulmonic insufficiency was noted in 9/10 dogs (90%), and

6/10 (60%) had trace to mild tricuspid regurgitation. In all cases, the degree of valvular regurgitation was considered hemodynamically inconsequential.

M-mode measurements

All measured indices of left ventricular systolic function on M-mode were significantly different on sotalol than at baseline. These included a reduction in FS (Figure 12) and an increase in LVIDs and EPSS (Table 2). Post-treatment LPWs was significantly lower than baseline by a median of 0.95 mm ($p = 0.004$). There was no significant difference between baseline and post-treatment LPWd, IVSd, IVSs, LVIDd, or TAPSE (Table 3) on M-mode measurements.

Dog	Age (years)	Weight (kg)	Temperature (ºF)	HR (bpm)	RR (breaths/min)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	Sotalol dose (mg/kg)
1	6.27	21.0	101.0	130	20	143	99	111	1.90
2	3.26	27.9	100.2	80	32	124	70	89	1.43
3	1.54	28.3	100.9	60	40	112	55	82	1.41
4	3.84	22.0	101.5	100	36	135	86	99	1.82
5	2.45	22.6	101.8	120	--	128	74	89	1.77
6	1.93	24.7	103.3	120	--	113	56	79	1.62
7	4.79	27.4	100.5	90	--	121	62	85	1.46
8	6.40	27.0	102.2	140	60	121	61	85	1.48
9	1.09	35.8	101.2	90	40	139	65	99	1.12
10	2.31	24.7	101.5	80	--	132	64	87	1.62
Mean	3.39	26.1		101		127	69	91	1.56

Table 1: Baseline physical examination and blood pressure data for 10 normal dogs. HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. Respiratory rate denoted -- in panting dogs.

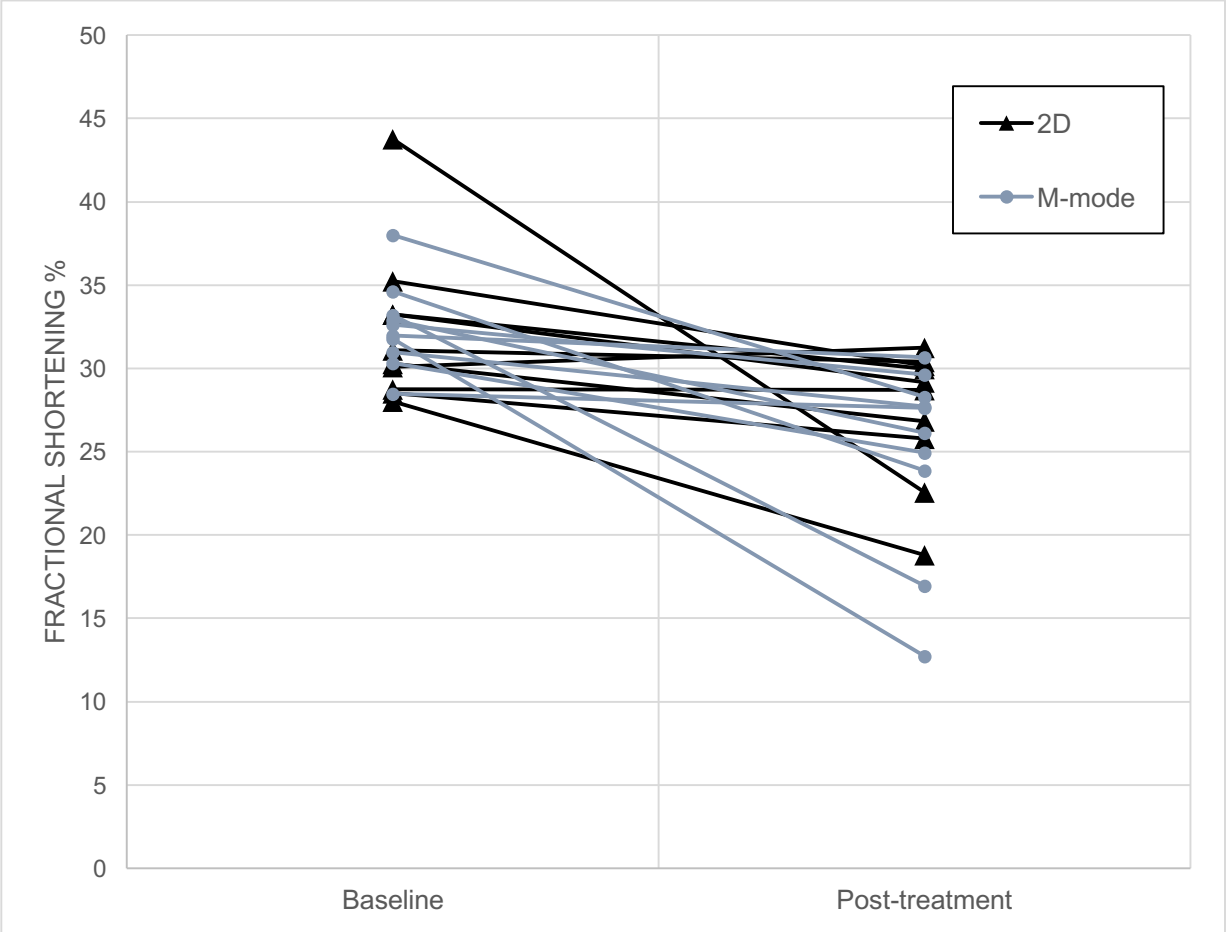

Figure 12: Fractional shortening with 2D and M-mode measurements at baseline and after treatment with sotalol in 10 normal dogs.

	Baseline	Post-treatment	Mean or median of differences	95% CI of mean/median of differences	*P* value
MM FS (%)	32.5 (2.6)	24.9 (5.7)	7.64	3.17 – 12.11	**0.004**
2D FS (%)	30.7 (28.7-33.8)	27.4 (4.0)	3.39	0.05 – 9.23	**0.010**
MM LVIDs (mm)	25.5 (2.4)	28.7 (3.2)	3.22	0.96 – 5.48	**0.010**
2D LVIDs (mm)	25.2 (2.9)	28.3 (2.0)	3.11	1.63 – 4.59	**0.001**
ESV (SMOD) (mm^3)	26.0 (4.6)	30.0 (4.6)	3.95	1.28 – 6.62	**0.009**
EF (SMOD) (%)	53.8 (4.4)	48.0 (6.8)	5.80	2.77 – 8.83	**0.002**
MM EPSS (mm)	3.08 (0.92)	3.99 (0.97)	0.91	0.25 – 1.57	**0.012**
Peak S' (lateral) (mm)	0.14 (0.02)	0.13 (0.02)	NS	NS	0.235
Peak S' (septal) (mm)	0.11 (0.02)	0.09 (0.01)	0.02	0.01 – 0.03	**0.003**

Table 2: 2D and M-mode (MM) echocardiographic indices of left ventricular systolic function at baseline and after treatment with sotalol in 10 normal dogs; data are presented as mean (standard deviation) for normally distributed data and median (interquartile range) for non-normally distributed data. NS, not significant; CI, confidence interval; FS, fractional shortening; LVIDs, left ventricular internal diameter at end-systole; ESV, end-systolic volume; EF, ejection fraction; EPSS, E point to septal separation.

	Baseline	Post-treatment	Mean or median of differences	95% CI of mean of differences	*P* value
FAC (%)	40.8 (9.4)	39.2 (6.3)	NS	NS	0.541
TAPSE (mm)	12.0 (3.1)	11.2 (2.8)	NS	NS	0.183
Peak S' (lateral) (mm)	0.13 (0.02)	0.11 (0.02)	NS	NS	0.069

Table 3: 2D and M-mode (MM) echocardiographic indices of right ventricular systolic function at baseline and after treatment with sotalol in 10 normal dogs; data are presented as mean (standard deviation) for normally distributed data and median (interquartile range) for non-normally distributed data. NS, not significant; CI, confidence interval; FAC, fractional area change; TAPSE, tricuspid annular plane systolic excursion.

2D measurements

All measured indices of left ventricular systolic function on 2D were significantly different on sotalol than at baseline. These included a reduction in FS (Figure 12) and EF (SMOD) (Figure 13), and an increase in LVIDs and ESV (SMOD) (Table 2). Post-treatment LPWs was significantly lower than baseline by a mean of 1.35 mm (95% CI: 0.57-2.13, p = 0.003). LVIDd was significantly increased by 1.93 mm (95% CI: 0.38-3.48, p = 0.020) on sotalol compared to baseline. There was no significant difference between baseline and post-treatment LPWd, IVSd, IVSs, EDV, LA/Ao, or RV FAC (Table 3) on 2D measurements.

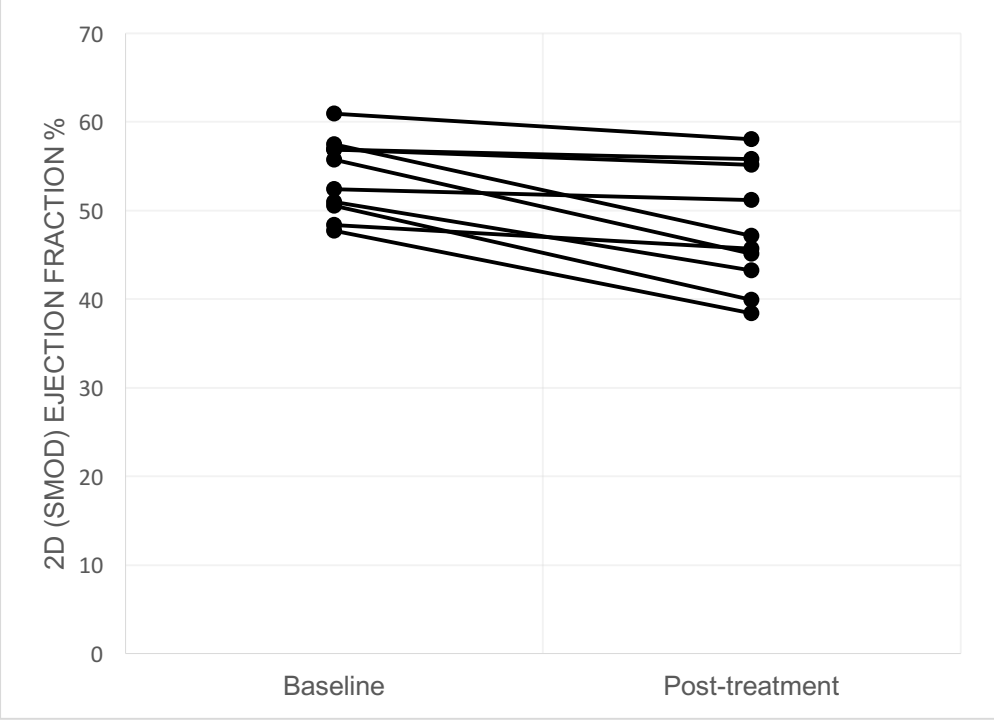

Figure 13: Ejection fraction measured on 2D images using Simpson's method of discs (SMOD) at baseline and after treatment with sotalol in 10 normal dogs

Spectral Doppler measurements

Peak pulmonic (PV) and aortic (AV) outflow velocities were both significantly (p = 0.004, 0.002, respectively) lower on sotalol (PV: 0.77 m/s ± 0.11; AV: 1.28 m/s ± 0.18) than at baseline (PV: 0.92 m/s ± 0.13; AV: 1.53 m/s, IQR 1.21-1.62). The E/A ratio was significantly (p = 0.016) higher on sotalol (1.81 ± 0.47) than at baseline (1.46 ± 0.32).

Tissue Doppler measurements

S' measured at the septal mitral annulus was significantly lower on sotalol than at baseline (Table 2). There was no significant difference in S' measured at the lateral mitral annulus (Table 2) or lateral tricuspid annulus (Table 3) before and after treatment. There was also no significant difference in E/E' (lateral mitral annulus) before and after treatment.

3D echocardiography

Technical issues with the picture archiving and communication system (PACS) resulted in the loss of several 3D studies; baseline and post-treatment studies from 7/10 dogs were available for interpretation. Review of the acquired 3D LV volume sets revealed universally poor image quality from the left apical view, and border detection was considered inaccurate and incomplete despite manual adjustment (Figure 6). Similarly, 3D RV volume sets, which were also acquired from the left parasternal view, were considered to be of poor quality. Therefore, only images from the right parasternal four-chamber view were included for analysis of ventricular function.

Real-time 3DE measurements included left ventricular EDV, ESV, SV, EF, GLS, GCS, twist, and torsion, none of which was significantly different before and after treatment with sotalol (Table 4, Figures 14, 15). Baseline and post-treatment 3D ESV,

EDV, and EF were compared to the corresponding 2D volumetric measurements using SMOD. There was no significant difference between the 2D and 3D measurements.

	Baseline	Post-treatment	Mean or median of differences	95% CI of mean of differences	*P* value
ESV (mm^3)	26.5 (16.5–36.5)	28.3 (23.5-34.9)	NS	NS	0.219
SV (mm^3)	29.2 (27.1-38.6)	31.0 (22.2-37.9)	NS	NS	0.938
EF (%)	52.5 (51.5-59.2)	51.1 (49.3-53.8)	NS	NS	0.375
GLS (%)	-20.2 (-21.5- -18.5)	-18.33 (-21- -16.7)	NS	NS	0.109
GCS (%)	-23.5 (-28.9- -20.2)	-23.0 (-24.2- -22.3)	NS	NS	0.469
Twist (º)	14.8 (7.1-16.7)	11.0 (8.8-12.1)	NS	NS	0.469
Torsion (º/cm)	2.10 (1.17-2.97)	1.60 (1.47-2.07)	NS	NS	0.469

Table 4: 3D echocardiographic volumetric and strain indices of left ventricular systolic function at baseline and after treatment with sotalol in 7 normal dogs; data are presented as median (interquartile range). NS, not significant; CI, confidence interval; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction; GLS, global longitudinal strain; GCS, global circumferential strain.

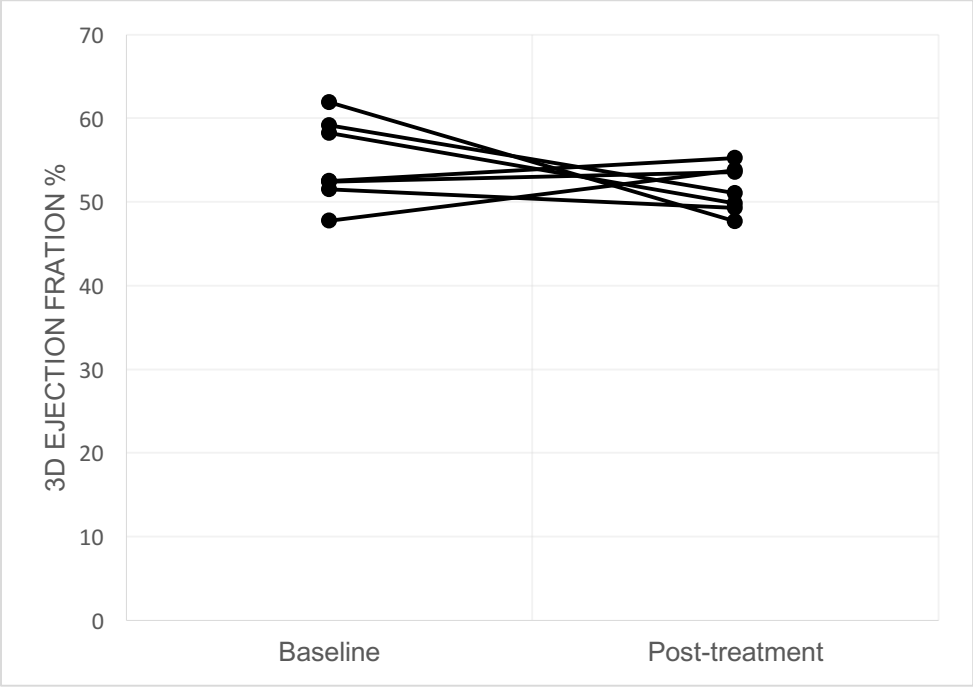

Figure 14: Ejection fraction measured on 3D images at baseline and after treatment with sotalol in 7 normal dogs.

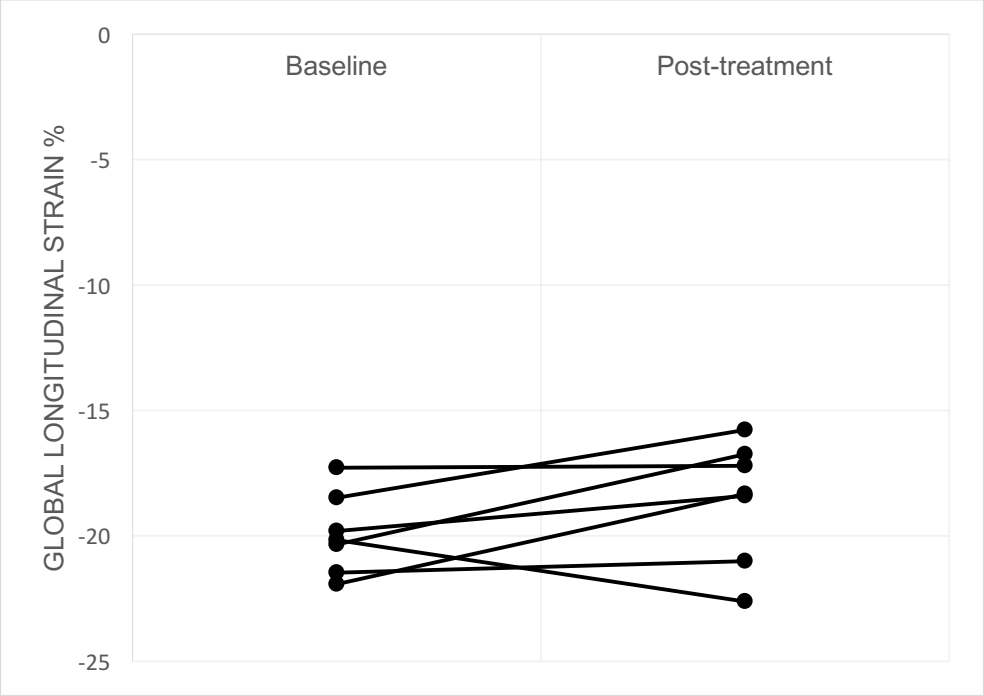

Figure 15: Global longitudinal strain measured on 3D images at baseline and after treatment with sotalol in 7 normal dogs.

Holter data

There was a median of 1.5 VPCs (IQR 0-3.5) and 3 pauses greater than 3 seconds (IQR 0-13) on baseline Holter. The baseline minimum heart rate was 34 ± 2 bpm, average heart rate was 64 bpm (IQR 61-68), and maximum heart rate was 218 bpm (IQR 211-224); only the maximum heart rate was significantly different post-treatment at 195 ± 14 bpm ($p = 0.002$) (Figure 16). There was no significant difference in the total number of VPCs post-treatment (median 0, IQR 0-3), nor in the number of pauses (median 4, IQR 0-14.75). One dog (case 9) had 150 single VPCs and 54 single APCs on baseline Holter, and 126 VPCs and 64 APCs post-treatment, although only sinus rhythm was noted on baseline ECG and during the echocardiogram.

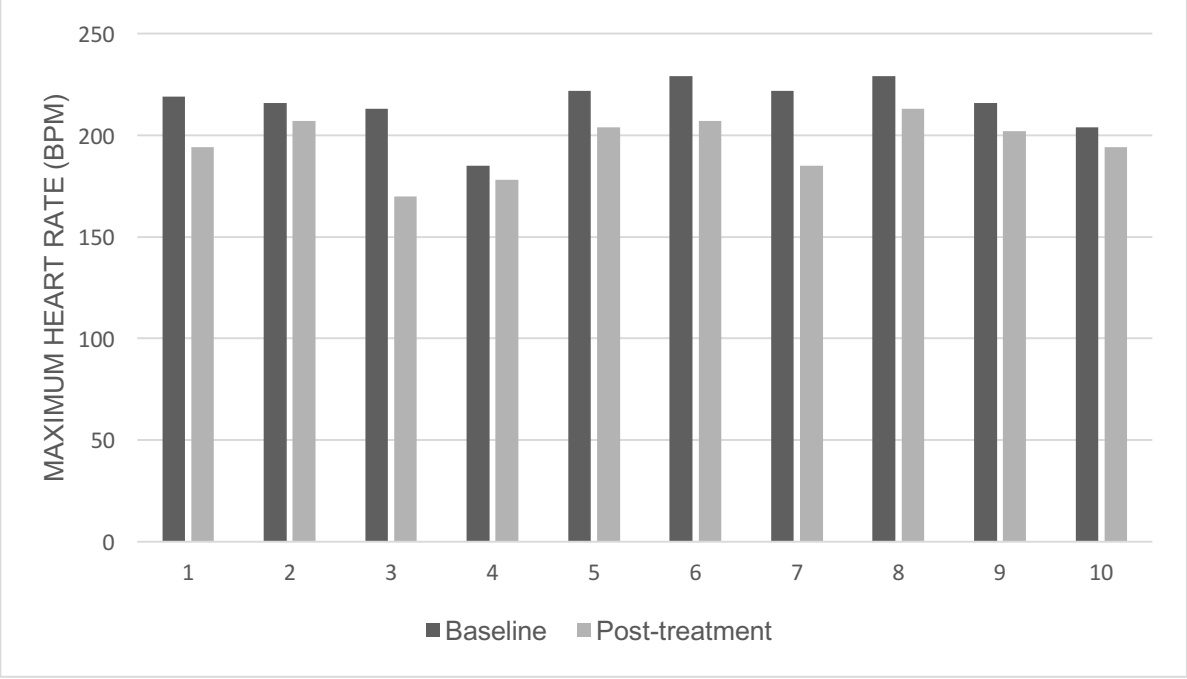

Figure 16: Maximum heart rate on 24-hour Holter monitor at baseline and after treatment with sotalol in 10 normal dogs.

DISCUSSION

Sotalol is an anti-arrhythmic drug used often in dogs with heart disease whose clinically relevant effects on ventricular function remain poorly characterized. This study has shown that sotalol has mild but significant negative inotropic and chronotropic effects in large breed, healthy dogs. Conventional echocardiographic techniques provided almost uniform evidence of a reduction in systolic function, with the exception of the peak lateral mitral annular systolic velocity (S'), including a mean reduction in M-mode fractional shortening of 7.6% and mean reduction in 2D ejection fraction of 5.8%. These findings are consistent with some of the experimental studies that have shown a reduction in systolic function in dogs from beta-blockade due to sotalol,[38,70,130] but in contrast to one study that did not identify a myocardial depressant effect of sotalol, including no change in the force-velocity curve from sotalol-induced beta blockade in the canine arm of the experiment.[67]

Although beta-blockade would be expected to influence right ventricular systolic function as well, no significant change was noted in the measured indices of right ventricular systolic function. The study may have been underpowered to detect small changes in these measurements, and nonvolumetric indices are likely relatively insensitive measures of right ventricular systolic function.[131] The goal of this study was to evaluate the effect of sotalol as it is most often used clinically, as chronic oral therapy. It is difficult to speculate on whether this small reduction in systolic function in normal dogs can be extrapolated to dogs with heart disease, especially given that the effect of sotalol may vary in the presence of heart disease.[47,64] However, even a small reduction in systolic function may have clinical importance in some disease states, and this should be considered before initiating sotalol.

The present study also showed a negative chronotropic effect of sotalol, although this was reflected in only the maximum heart rate on Holter monitor and the heart rate on physical examination, with no significant difference in the mean or minimum heart rates, or the number of pauses noted. This may reflect a more significant influence in conditions where sympathetic tone is high, such as in the hospital or during periods of excitement or activity at home. However, this is in contrast to a study in Boxers with ventricular arrhythmias where 16 dogs treated with sotalol showed a reduction in minimum, mean, and maximum heart rates on Holter monitor.[10] It is possible a larger number of dogs would have yielded detection of a smaller difference between groups.

Sotalol's apparent negative inotropic effect is often attributed to direct influence of beta-blockade, but some part of the reduction in systolic function may be related to a reduction in contractility at slower heart rates due to the force-frequency relationship.[54] However, an experimental study in dogs suggests that sotalol blunts the force-frequency response based on abolishment of the inverse relationship between cycle length and left ventricular peak dP/dt seen at baseline.[46] In fact, it showed a positive inotropic effect relative to baseline at very slow heart rates; at cycle lengths relevant to the present study, LV dP/dt was similar to baseline and significantly better than with esmolol.[46] It is important to consider that this experimental study was performed in anesthetized dogs that were likely experiencing a marked reduction in sympathetic outflow,[132] which might mitigate the effects of drug-induced beta-blockade relative to an awake dog. Therefore, the blunting of the force-frequency response (which was correlated with the increase in APD) and positive inotropic effects may be overcome by the influence of beta-blockade in an awake dog. A similar experimental protocol in sedated humans with chest pain did show a negative inotropic effect with some preservation of the force-frequency response.[74] The relative contributions of direct antagonism of beta-receptors and a

slower heart rate to a reduction in systolic function in this study is unknown. Because the force-frequency relationship becomes flat or negative at higher heart rates in heart failure,[133] this relationship might be expected to be altered in dogs with heart disease.

The demonstrated negative inotropic and chronotropic effects of sotalol support a reduction in myocardial oxygen demand, which may be desirable in dogs at risk for ischemia (i.e. those with pressure hypertrophy) or in heart failure. This reduction in myocardial oxygen demand has been demonstrated experimentally in dogs.[24] Combined with its class III anti-arrhythmic effects, these findings argue that sotalol may prove a more effective medical therapy for dogs with subaortic stenosis despite the historical preference for drugs with predominantly class II effects. For similar reasons, sotalol might also be considered to treat hypertrophic cardiomyopathy (HCM). Feline HCM is often treated with atenolol, especially in the presence of left ventricular outflow tract obstruction, although this has failed to demonstrate a long-term benefit.[134,135] In humans, medical therapy, including sotalol and beta-blockers, does not protect against sudden cardiac death but may reduce the occurrence of ventricular arrhythmias.[7] Canine HCM is uncommon but shares morphologic similarities to HCM in cats and humans and may benefit similarly from the negative inotropic, chronotropic, and anti-arrhythmic effects of sotalol.[136,137]

This study suggests that caution is warranted for sotalol use in those patients that are hemodynamically unstable and may not tolerate a reduction in ventricular systolic function. As a well-tolerated and efficacious anti-arrhythmic, sotalol is often considered in dogs with dilated cardiomyopathy and arrhythmogenic cardiomyopathy (ARVC) in whom arrhythmias are common.[10,138] However, systolic dysfunction is a prominent feature of DCM and is occasionally present in Boxers with ARVC, and therefore the negative inotropic and anti-adrenergic effects of sotalol might be

considered detrimental with advanced disease.[82,127,139,140] These effects may be mitigated by a more potent increase in APD with sotalol in dogs with heart disease and an increase in contractility at lower heart rates due to the negative force-frequency relationship in heart failure.[46,64] However, sotalol's effects on systolic function remain to be determined in dogs with naturally occurring heart disease or heart failure. Therefore, as slow up-titration of beta-blockers is recommended to minimize acute clinical deterioration in dogs with DCM, this should also be considered when initiating sotalol in these patients.[18,141] Future studies should evaluate whether beta-blockers, including those with other class effects like sotalol, have a role in the treatment of common acquired canine heart diseases with or without arrhythmias.

None of the three-dimensional measurements in the present study changed significantly with sotalol therapy, but the interpretation of these data is limited by the lack of experience with 3DE in veterinary medicine. There are three studies in dogs on volumetric assessment of the left ventricle using RT3DE compared to MRI or MDCT as the reference standard. MRI is considered the gold standard in humans, but it has shown excellent correlation with MDCT in humans and dogs.[83,142,143] One of the veterinary validation studies showed a strong correlation between RT3DE and CMR measurements of EDV and ESV, but no correlation between EF measured using RT3DE and CMR;[115] another showed similarly strong correlation between RT3DE and MDCT measurements of EDV and ESV and weak correlation for EF.[83] A third study evaluated two different types of software versus CMR, including software from the same vendor (TomTec) as that used in the results reported here; however, manual contouring was used for the TomTec analysis in those dogs. That study reported a moderate correlation for EDV measured using RT3DE (4D-TomTec) and CMR, but no correlation for ESV, SV or EF; no correlation between measurements obtained using the other software (4D-

AutLVQ) and CMR were noted for any of the volumetric measurements.[116] These studies suggest that, with the exception of ESV, indices of function such as EF and SV may not be accurately reflected by RT3DE. These findings are in contrast to those in human medicine, where EF based on RT3DE shows good agreement with CMR, despite underestimation of left ventricular volumes.[113,144,145]

While 3DE is superior in reproducibility and repeatability than 2DE based on human studies, there remains substantial variability, especially within individuals, that must be considered when monitoring a single patient over time.[113,144,145] Repeatability of 3DE left ventricular volumes in dogs appears acceptable relative to 2DE,[116,146,147] but interobserver variability has been reported only once with several measures showing significant variability.[116] RT3DE underestimates LV volumes in both human and veterinary medicine,[83,113,115,116,144,145] although the bias in dogs between RT3DE and contrast-enhanced MDCT was very small,[83] and data from humans suggests this bias may be reduced with experience.[145] The reason for this underestimation most likely reflects errors in tracing the endomyocardial border related to insufficient spatial resolution; even a barely perceptible shift of the trace surface 1 mm can have a substantial effect on the measured volume.[144]

The lack of significant difference in 3DE measures of systolic function in dogs before and after treatment with sotalol in the present study may be due to a lack of power, given the limited number of dogs for whom 3DE data were available and individual variability. If, as suggested by some of the validation studies, RT3DE provides an inaccurate estimate of systolic function indices in dogs, this may simply reflect the inaccuracy inherent in RT3DE; indeed, one study showed stronger correlation for ESV and EF measured with 2DE SMOD from the right parasternal view compared to CMR than for RT3DE and CMR.[115] An alternative explanation is that the significant differences

found with conventional methods are erroneous and that RT3DE accurately showed no change with sotalol therapy. It is important to note that the left ventricular volumes measured using RT3DE in this study were obtained from the right parasternal four-chamber view rather than the left apical view, and as such cannot be directly compared to measurements reported in the literature.

In humans, breath holding is recommended to minimize respiratory artifact in multi-beat RT3DE acquisition, and most studies performed have excluded patients with arrhythmias, which can also lead to suboptimal image quality.[113,148] A meta-analysis revealed that 7.5% of patients were excluded for suboptimal images (of those that reported exclusions), and one study reported 20% of patients had technically difficult images.[113,149] Reported variability is often based on optimal image quality and therefore may not reflect variability in practice. In dogs, MDCT and CMR as reference standards necessitates anesthesia for direct comparison with echocardiography, and in one of the reported studies esmolol was used for heart rate control.[83] These conditions allow for better image quality, especially important in multi-beat acquisition of RT3DE images, by avoiding the conditions faced in clinical practice with awake dogs including poor patient compliance, respiratory artifact, and arrhythmias, all of which were negotiated in our study.

The authors found that imaging from the left apical view in this cohort of healthy, unsedated, large breed dogs led to suboptimal quality for RT3DE volume estimates compared to the right parasternal views. This may reflect improved image quality and less technically demanding image acquisition from the right side, especially in large, deep-chested dogs.[85,150] One study of RT3DE obtained from the left apical view in unsedated dogs with degenerative valve disease showed good intraobserver acquisition and measurement variability for global LV volumes, however, these dogs were all under

15 kg.[147] Another study including RT3DE in dogs with and without heart disease, representing a larger variety of breeds and body weights, showed higher but still acceptable coefficients of variation.[146] Neither of these studies had a reference standard for comparison to determine accuracy. Notably, the large majority of the dogs used in the veterinary validation studies were Beagles, which have a smaller body size and slightly broader chest conformation than most of the dogs in this study, possibly also contributing to better image quality from the left apical view.

The right parasternal four-chamber view has shown good agreement with the left apical view for volumetric measurements using 2DE.[84-86] Apical foreshortening in this view is not a concern in 3D imaging because 3DE obviates the need for geometric assumptions based on a single plane, whereas image quality and border detection is paramount. Based on the experience in this study and because good-quality echocardiographic images can be more universally obtained from the right parasternal view in a wider range of dogs, this may be a preferable view for routine 3DE evaluation of the left ventricle in clinical practice. Future studies to validate RT3DE of the left ventricle from the right parasternal view should be considered.

3DE speckle-tracking echocardiography for deformation analysis is a nascent technology that has not been reported in veterinary medicine to date. This study reports 3D STE global longitudinal strain, global circumferential strain, twist, and torsion in a small number of normal, large breed dogs. There are no validation studies in veterinary medicine nor normal reference ranges, and therefore the clinical significance of these findings is unclear. However, these measurements are calculated automatically from a full-volume left ventricular RT3DE data set and can be easily obtained from a single acquisition. After off-line analysis to generate an LV cast, no further manual measurements are needed. This method may be more feasible in clinical practice for

strain measurement than 2D STE or TDI, especially as 3DE becomes more widely available.

The limitations of the present study are primarily related to the small number of dogs, and therefore the study may have been underpowered to detect changes in indices of right ventricular function or to identify changes in the 3DE measurements. Additionally, echocardiography is not the most accurate way to measure systolic function. However, the goal of this study was to assess systolic function in awake and unsedated dogs as a more clinically relevant measure of sotalol's effects, precluding advanced imaging or cardiac catheterization. Another limitation is the use of echocardiographic techniques that are unvalidated in veterinary medicine; however, these are presented only as feasible and not considered to have clinical relevance in this study. In addition, the relative impact of heart rate on contractility was not evaluated for each dog, as discussed, and this may be a confounding factor masking the direct effects of the drug. One dog had evidence of clinically significant arrhythmias of unknown cause noted only after Holter analysis. This dog was included in the study because the heart was structurally unremarkable echocardiographically at the time of inclusion, but could conceivably have had myocardial dysfunction that was inapparent and thus may not represent a normal control. Similarly, a dog with mild degenerative valve disease was included based on the lack of chamber enlargement and only trace to mild valvular regurgitation, but very mild dysfunction or remodeling secondary to volume overload cannot be entirely excluded in this dog.

Conclusions

Sotalol has a mild negative inotropic effect in healthy, large breed dogs based on conventional two-dimensional echocardiography, and a negative chronotropic effect is

present at high heart rates. Three-dimensional left ventricular volumetric and deformation analysis is feasible from the right parasternal four-chamber view in awake, large-breed dogs, and may provide superior image quality to that obtained from the left apical view.

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