INVESTIGATION AND ASSESSMENT OF EJECTION MURMURS AND THE LEFT VENTRICULAR OUTFLOW TRACT IN BOXER DOGS

DISSERTATION

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By

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ABSTRACT

Aortic stenosis (AS) is a common, heritable congenital heart defect affecting Boxer dogs. Severely affected dogs are at risk for heart failure, endocarditis, and sudden death. Veterinarians usually screen for AS through auscultation. When a typical murmur is auscultated, an echocardiogram is performed to identify the presence and severity of the cardiac defect. In the setting of a soft murmur, echocardiographic imaging lesions are often absent, and a noninvasive diagnosis is based on Doppler-identification of high velocity flow across the left ventricular outflow tract (LVOT). However, similar increases in LVOT velocity can occur in normal dogs under sympathetic stimulation. This distinction between mild AS and normal variation is pivotal to Boxer breeders.

In a review of 201 healthy adult boxers, over 50% had left basilar ejection murmurs typical of AS, and the combination of a murmur and elevated LVOT velocity was seen in 36%. For those dogs, the presence of a murmur predicted an LVOT velocity > 2 m/s, with an 86.9% sensitivity and 65.8% specificity. These findings validate the standard practice of screening dogs with ausculation prior to performing an echocardiogram, a more expensive test.

Detailed echocardiographic assessment of the LVOT morphology demonstrated that indexed diameters and areas were not different between boxers with ejection murmurs and boxers without murmurs, although they were smaller than in non-boxer dogs. Calculated stroke volume, by echocardiography, showed a trend for boxers with murmurs to have higher stroke volumes. These data indicated that a smaller LVOT may predispose all boxers to an ejection murmur and elevated ejection velocity, while the stroke volume helps determine the presence or absence of these findings in any particular dog.

Angiographic assessment of the LVOT morphology also demonstrated a diffusely smaller LVOT in boxers with murmurs compared to non-boxer dogs. With the exception of the mid-portion of the ascending aorta, all indexed LVOT diameters were smaller in boxers. Both subjective and objective evidence supported substantial relative narrowing at the sinus of Valsalva. Boxer dogs also showed a trend toward higher catheterization-derived pressure gradients across the LVOT, but only when the stroke volume was sufficiently increased during dobutamine infusion. Significant peak to peak gradients were not found in most boxers, and subjective interpretation of the pressure waveforms strongly favors an impulse gradient. These data further support the influence of both a diffusely narrowed LVOT and an elevated stroke volume underlying ejection murmurs and elevated ejection velocities in boxer dogs.

Intracardiac phonocardiography indicated that left basilar ejection murmurs develop within the distal LVOT, either at the aortic valve or within the

proximal ascending aorta. This finding helps exclude the possibility of occult subvalvular obstruction and is consistent with valvular stenosis, supravalvular stenosis, a elevated stroke volume, or most likely a combination of these factors.

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FIELDS OF STUDY

Major Field: Veterinary Clinical Sciences

Studies in Veterinary Cardiology

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CHAPTER 1

INTRODUCTION

Aortic stenosis (AS), typically subvalvular aortic stenosis, is the second most common congenital heart defect in dogs, and the reported incidence among boxers is particularly high,[1;2;3] with this breed comprising 40% of all cases of AS in one study.[4] The actual prevalence of AS in the boxer population is not known, but cardiac murmurs consistent with AS have been observed in over 50% of healthy adult boxers.[4;5;6] The majority of these murmurs have been found to be soft left basilar systolic murmurs (ejection murmurs) graded as either 1 or 2/6.[4;6] Since aortic velocities (AV) have been found to roughly correlate with murmur intensity,[7] these findings suggest that although the prevalence of AS in boxers may be high, the majority of the affected animals have mild disease. Boxers diagnosed with "mild AS" based on Doppler velocity findings generally have normal outflow tract anatomy by echocardiographic assessment,[7;8] and some investigators have suggested the presence of left ventricular outflow tract (LVOT) obstruction at the valve or supravalvular level instead of at the subvalvular region.[9;10] Although supravalvular and valvular aortic stenoses are thought to be relatively uncommon in dogs,[11;12] one author

has suggested that aortic hypoplasia (in the absence of pathologic stenosis) may be associated with the development of an ejection murmur in the boxer dog.[10] This observation could be associated with a mismatching of left ventricular volume with LVOT area as described by Pasipoularides.[13]

Upon physical examination, cardiac murmurs related to stenosis or hypoplasia of the LVOT are similar in timing and location to physiologic murmurs, which are not associated with a pathologic cardiovascular abnormality. Adult humans and dogs can develop physiologic murmurs in association with high cardiac output states or decreased blood viscosity, as with anemia, fever, hyperthyroidism, and pregnancy.[14;15] The development of physiologic murmurs is also seen in athletic humans,[16;17] dogs,[18] and horses[19] at rest, and in normal non-athletic individuals following short periods of exercise.[15] Cardiac murmurs were detected in 40-45% of heavily trained Alaskan sled dogs.[18;20] Although Doppler echocardiograms were not performed on these dogs, the authors of the study felt that the murmurs represented a physiologic response rather than an indicator of cardiac disease, as the dogs with murmurs were subjectively better athletes and all were capable of running for long distances.[20] Many authors have previously speculated about the presence of 'innocent' murmurs in adult dogs and the possibility that boxers have physiologic instead of pathologic heart murmurs.[5;6;7;8]

Unfortunately, auscultation cannot differentiate between mild LVOT obstruction and a physiologic murmur. Even when auscultation is aided by the use of trans-thoracic phonocardiography to visualize and evaluate the

frequency components of the murmur, it is difficult to reliably separate dogs with mild AS from normal dogs with physiologic murmurs. [5;7]

Historically, the diagnosis of AS was made by cardiac catheterization including angiography and direct pressure recordings of the LVOT. Angiography was performed to outline the anatomy of the LVOT in order to identify and confirm a region of stenosis, as well as evaluate the presence of secondary effects, such as left ventricular concentric hypertrophy and post-stenotic dilation of the ascending aorta. Direct pressure recordings were used to determine the presence and magnitude of an increased pressure gradient (PG) across the LVOT. Despite the utility of echocardiography, cardiac catheterization with direct pressure measurements remains the gold-standard test for determining the hemodynamic impact of a stenotic lesion. The gold-standard test for the assessment of stenotic morphologic lesions is unavailable. Current ultrasound technology likely makes echocardiography more sensitive than angiography, although comparisons between these techniques are uncommonly evaluated.

For most clinical patients, however, cardiac catheterization has largely been replaced by Doppler echocardiography since Doppler-derived PGs have been demonstrated to correlate well with direct pressure measurements in dogs with moderate to severe AS.[21] Doppler echocardiography has the additional benefits of being non-invasive and can be performed in the awake dog.

Normal canine Doppler blood flow velocities in the LVOT have been documented, and the corresponding calculated PGs are used as reference

points to diagnose an increased PG observed in some boxers with soft heart murmurs. The upper limit of the reference range is 1.5 – 1.7 m/s for normal dogs using the left apical echocardiographic plane. [22-25] However, it has been shown that the subcostal imaging plane allows more parallel alignment with the aortic outflow, and this is now the preferred method of measuring aortic velocities.[26] Although reference ranges using the subcostal position had not been established in dogs at the onset of this project, velocities less than 1.7 m/s were generally considered normal. [27] At that time, reported upper limits of normal aortic velocities ranged from 2.0 – 2.5 m/s in the veterinary literature.[1;5;9;25;28] In 2003, subcostal aortic velocities from 49 dogs (breed unknown) were reported to be between 1.18 and 1.99 m/s.[29] These recent findings supported the use of an upper limit of 2 m/s for the subcostal position.

The hypothesis of this project was that boxer dogs with ejection murmurs and increased aortic velocities have aortic stenosis. The specific aims of this project were to determine the relationship between AV and murmur intensity and the variation in these clinical findings over time; to provide a thorough structural and functional evaluation of the LVOT by echocardiography in boxers with and without soft ejection murmurs; to measure pressure gradients during cardiac catheterization and under the influence of flow patterns that may be observed with physiologic murmurs; and to localize the site of murmur generation within the LVOT using intracardiac phonocardiography.

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CHAPTER 2

AORTIC EJECTION VELOCITY IN BOXER DOGS

Introduction

Low-grade, left-basilar ejection murmurs are frequently recognized in boxer dogs, with at least 50% of dogs at European breed shows demonstrating this type of cardiac murmur.[1-3] These murmurs are presumptively related to increased aortic ejection velocity,[1,4] which may be associated with obstructive or physiologic flow disturbances within the left ventricular outflow tract (LVOT). Aortic stenosis (AS), typically subvalvular, is the most common form of LVOT obstruction among boxer dogs, with reported odds ratios of relative risk ranging from 5.4 to 8.6.[5,6] Alternatively, aortic ejection murmurs in healthy dogs may represent undefined physiologic flow disturbances in the LVOT, associated with sympathetic tone,[1,7] athleticism,[8] or anatomic factors (such as body conformation or size/shape of the aorta relative to the proximal LVOT).[9,10]

Determining the correct etiology of these murmurs has a major impact on breeding decisions, as congenital AS is considered a heritable defect.[11-13] It is also relevant to the pet owner, as dogs with mild AS carry an increased risk of

bacterial endocarditis,[6] a condition that may be life-threatening. To investigate the scope of this diagnostic dilemma, we retrospectively evaluated the auscultatory and Doppler echocardiographic findings in a large group of boxers. The objective of this study was to define the aortic velocity and murmur grade in healthy boxer dogs without overt structural evidence of LVOT obstruction. A secondary aim was to describe the within-dog variation of these variables over time.

Materials and Methods

Criteria for Selection of Cases

Two hundred twenty-eight mature client-owned boxer dogs were evaluated as part of a prospective study of boxer cardiomyopathy between January 1997 and December 2001. Records were reviewed retrospectively, and 201 dogs met the following criteria. Boxers at least one year of age and without overt evidence of AS were considered potential subjects. For the purposes of this study, 'overt evidence of AS' was defined by a loud (> grade 3/6) systolic murmur or an obvious structural abnormality within the LVOT on a right parasternal long axis echocardiographic study. Only dogs with an ejection murmur loudest over the aortic valve area or at the left base and those without a murmur were included. Dogs with known systemic disease and those medicated with beta-adrenergic antagonists or L-thyroxine were excluded. No attempt was made to evaluate specific family groups, although a number of dogs in this study were related.

Procedures

A cardiovascular examination was conducted by two veterinarians experienced in the assessment of cardiac disease. Auscultation was performed in a quiet room while the dog was gently restrained. Intermittently both nostrils were occluded for 3-5 seconds to decrease the likelihood that respiratory noises might obscure soft cardiac murmurs. The absence or presence of a heart murmur was noted and graded by each examiner without knowledge of the other's results. The following classification scheme was used: grade 0/6 – no murmur was detected by either examiner after prolonged (at least 1 minute of) cardiac auscultation; grade 1/6 – a soft, intermittent ejection murmur was heard, or a soft, localized murmur was identified, but only after prolonged auscultation; grade 2/6 – a soft but localized murmur was immediately evident to the examiner; grade 3/6 – a moderately intense murmur was detected radiating over more than one valve area.[14] When the recorded murmur grade differed between examiners, auscultation was repeated by each until a consensus was reached.

All dogs underwent Doppler echocardiographic examination of the left ventricular outflow tract and ascending aorta. Dogs with cardiac murmurs also underwent standard 2D and color-flow Doppler echocardiography of the left ventricular inflow and outflow tracts. Maximal aortic velocity was determined using continuous-wave Doppler echocardiography recorded with a simultaneous electrocardiogram. Dogs were examined as they laid on a padded echocardiograph table, manually restrained in right lateral recumbency.

No sedatives or tranquilizers were administered for these examinations. A Vingmed System V color-flow echocardiograph system[a] with a 2.5 MHz transducer was used to record aortic velocities from the subcostal position.[15] The cursor was aligned parallel to flow using concurrent 2-D and color-flow Doppler echocardiography, and optimal signal strength was obtained by observing the real time spectral display and listening to the audio signal of the Doppler system. Doppler velocity spectra were recorded as digital files onto a magneto-optical disk and measured off-line with electronic calipers on an integrated workstation.[b] Three to five aortic velocity spectra with clear envelopes and well-defined peaks were identified and measured. The results were averaged to determine the maximal instantaneous aortic velocity.

Following initial evaluation, the clients were asked if they would return with their dog to permit reexamination on an annual basis. Forty-eight dogs were reevaluated in the same manner as outlined above, and the results of auscultation and Doppler echocardiography were compared between the baseline and follow up examination. For those dogs examined more than twice (4 dogs), the follow up examination used for comparison was that in which the greatest difference in aortic velocity was identified.

Statistical analysis

Measured cardiac variables were grade of cardiac murmur and maximal instantaneous (peak) aortic velocity. Body weight, age, and sex were also recorded. For purposes of statistical evaluation, dogs were grouped according to the absence (Group I) or presence (Group II) of a cardiac murmur and

analyzed within two aortic velocity groups (≤ 2.0 m/s and > 2.0 m/s). The aortic velocity of 2.0 m/s is an arbitrary value, however, it is based on previously-published recommendations for determining normal and abnormal aortic ejection velocities in dogs.[6,13,16-19]

A Mann-Whitney test was used to compare age and aortic velocity between male and female dogs, to compare age and aortic velocity between dogs with and without cardiac murmurs, and to compare murmur grade between the two aortic velocity groups. A Student's t-test was used to compare weight between male and female dogs and among dogs with and without murmurs, to compare follow up age and weight between dogs that changed auscultatory grouping (I vs. II) and those that remained unchanged, and to compare follow-up age and weight between dogs that changed aortic velocity classification (≤ 2.0 vs. > 2.0 m/s) with those that were unchanged. The associations between aortic velocity and age, body weight, and murmur grade were determined using Pearson's product-moment correlation or Spearman's rank correlation. Associations between change in murmur grade, change in aortic velocity, change in age, and change in body weight were similarly assessed. A Kruskal-Wallis ANOVA was used to compare aortic velocities across the different grades of murmurs. When significant differences were detected, Dunn's test was used for pairwise comparison. A paired t-test was used to identify differences between the peak aortic velocity measured at baseline and at the time of follow up examination. Statistical calculations were

performed by a computer software program,[c] and an alpha error of 0.05 was chosen as statistically significant for all analyses.

Results

Of the 201 dogs evaluated, 80 (40%) were male, and 121 (60%) were female. The mean age was 4.4 years (range: 1 – 12.7 years; median of 3.9 years), and body weight averaged 28.2 kg (62 lbs; range: 15.9 – 42.7 kg, 35 – 94 lbs; median of 27.7 kg, 61 lbs). The mean weight for male dogs was significantly greater than that for females (31 kg vs 26 kg, 68 vs. 57 lbs; $p < 0.001$). There was no gender difference in median age ($p = 0.7$).

The auscultatory and Doppler echocardiographic findings for each dog are displayed in Figures 2.1 and 2.2. The median maximum instantaneous aortic velocity for all exams was 1.91 m/s (range: 1.31 – 4.02 m/s; mean of 2.0 m/s). Median aortic velocities were not significantly different between males (1.96 m/s) and females (1.87 m/s; $p = 0.1$). A weak positive correlation was observed between aortic velocity and body weight ($r = 0.16$; $p = 0.03$). No significant correlation between aortic velocity and age was found ($r = -0.14$; $p = 0.06$).

Eighty-eight dogs (44%) did not have a detectable heart murmur (Group I), and 113 (56%) manifested a soft (grade 1-3/6), left-basilar ejection murmur (Group II). Group I was composed of 27 males (31%) and 61 females (69%), while group II consisted of 53 males (47%) and 60 females (53%). Group I

dogs were significantly older (median of 4.3 years) than those in Group II (median of 3.2 years; $p<0.001$). A significant difference in body weight was not detected between dogs with and without murmurs ($p = 0.7$), although the power for this test was low (0.05).

The median aortic velocity for Group I dogs was 1.72 m/s (range: 1.31 – 2.38 m/s; mean of 1.75 m/s). The median aortic velocity for Group II dogs was 2.11 m/s (range: 1.57 – 4.02 m/s; mean of 2.20 m/s), which was significantly higher than that for Group I dogs ($p<0.001$). The aortic velocity exceeded 2.0 m/s in 11 of the Group I dogs (13%), compared to 73 of the Group II dogs (65%; Figure 2.2).

Murmur grade was positively correlated with aortic velocity ($r = 0.67$, $p < 0.001$; Figure 2.1). Median aortic velocities for murmur grades 0/6 and 1/6 were significantly different from median aortic velocities of grades 2/6 and 3/6 ($p<0.05$). However, the median aortic velocity for grade 0/6 was not different than that for grade 1/6, and the median aortic velocity for grade 2/6 was not different than that for dogs with grade 3/6 murmurs ($p > 0.05$; Figure 2.1). The median murmur grade for exams with aortic velocity $\leq$ 2.0 m/s was significantly lower than the murmur grade for an aortic velocity > 2.0 m/s (grade 0/6 vs. 2/6; $p < 0.001$). Auscultation of a murmur was 86.9% sensitive, but only 65.8% specific, in the identification of an aortic velocity > 2.0 m/s. Of the 201 dogs, 73 (36%) demonstrated both a systolic ejection murmur and an aortic velocity over 2.0 m/s.

Forty-eight dogs underwent follow up examination, including 26 Group I dogs (54%) and 22 Group II dogs (46%). The median time between the initial and follow up examination was 13.5 months (range: 12 to 31 months; mean of 16 months). At the time of follow up evaluation, 22 of the Group I dogs (85%) remained murmur-free, while 4 (15%) demonstrated a left-basilar ejection murmur. Twelve of the Group II dogs (55%) continued to have a murmur on follow up exam, however a murmur was not detected in 10 of the dogs (45%) that demonstrated a murmur on initial examination. There was a non-significant trend for the Group I dogs that had a murmur on follow up evaluation to be younger than those that continued to have no auscultable murmur (3.6 years vs. 6.6 years, $p = 0.053$). The maximum individual changes in murmur grade were a decrease from grade 3/6 to 0/6 and an increase from grade 0/6 to 2/6. The change in murmur grade was not correlated with the change in age between examinations ($p = 0.93$) or the change in body weight ($p = 0.11$).

At initial examination, 36 dogs (75%) demonstrated an aortic velocity $\leq$ 2.0 m/s, and the aortic velocity for 28/36 (78%) remained $\leq$ 2.0 m/s at the follow up evaluation. The aortic velocity exceeded 2.0 m/s in 8 of those dogs (22%) with an average increase of 0.31 m/s (range: 0.15 to 0.59 m/s). The largest individual increase in aortic velocity was from 1.84 to 2.43 m/s. Twelve dogs (25%) had an initial aortic velocity over 2.0 m/s, and 6 of those dogs (50%) still had an aortic velocity > 2.0 m/s at the follow up exam. The aortic velocity decreased to < 2.0 m/s for the remaining 6 dogs, and the average decrease was 0.21 m/s (range: 0.07 to 0.39 m/s decrease). The largest individual

decrease was from 2.07 to 1.68 m/s. There was a non-significant trend for the dogs that had an initial aortic velocity > 2.0 m/s and a follow up aortic velocity ≤ 2.0 m/s to be older than dogs with both aortic velocities > 2.0 m/s (6.6 years vs. 4.1 years, p = 0.082). The overall mean aortic velocity at the follow up visit was significantly greater than that of the first visit (1.87 vs. 1.81 m/s, p = 0.04), however the actual difference is not clinically relevant (Figure 2.3). The change in aortic velocity was not correlated with the change in age between examinations (p = 0.18) or the change in body weight (p = 0.65). A positive correlation was detected between the change in murmur grade and the change in aortic velocity (r = 0.45, p = 0.002), however considerable individual variation was observed (Figure 2.4).

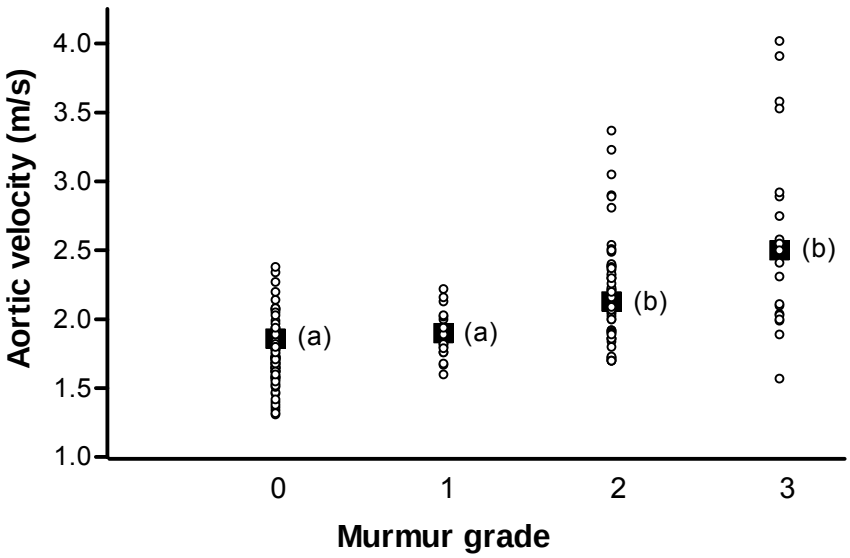

Figure 2.1: Relationship of Murmur Grade to Aortic Velocity.

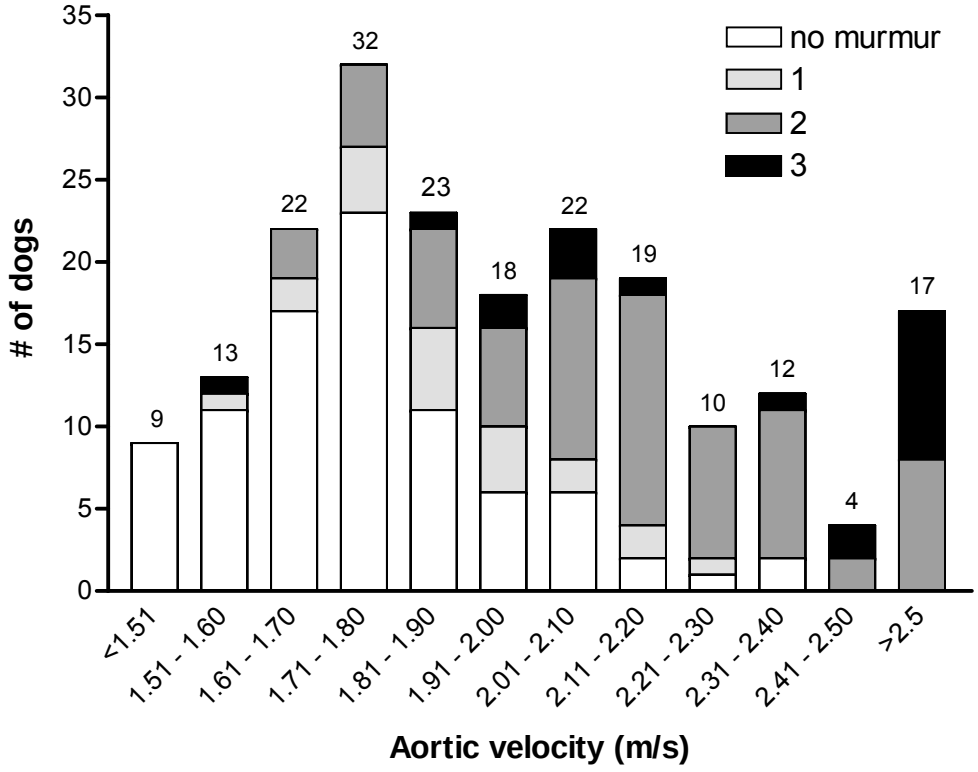

Figure 2.2: Frequency of Cardiac Murmurs Across Different Aortic Velocities.

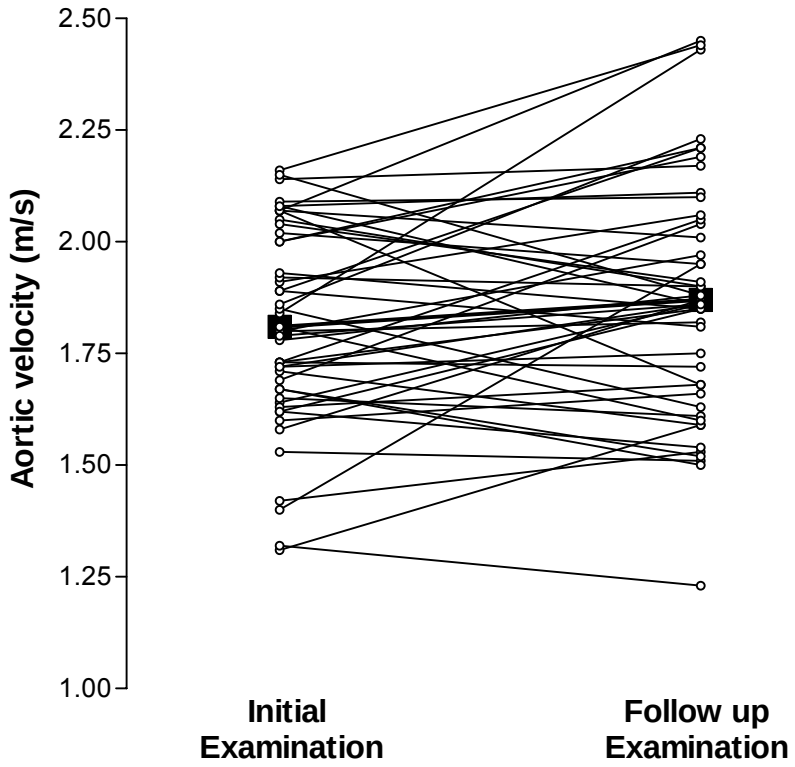

Figure 2.3: Change in Aortic Velocity.

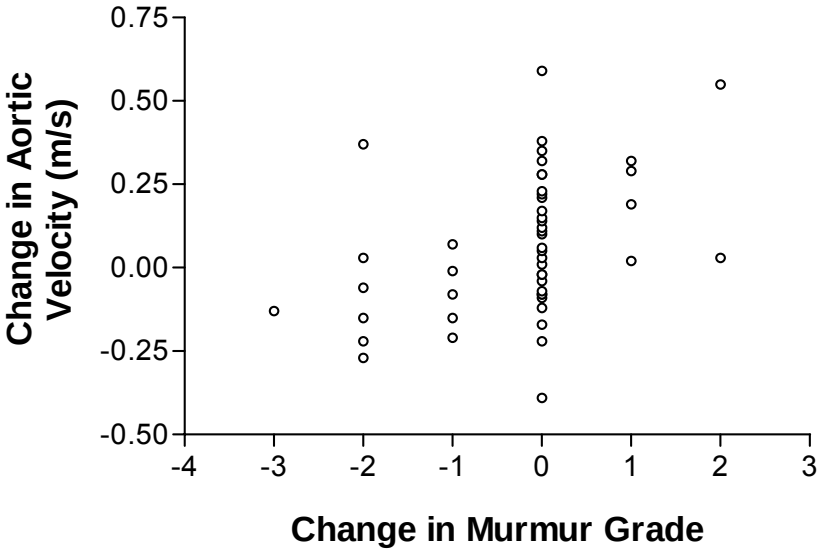

Figure 2.4: Effect of Change in Murmur Grade on Change in Aortic Velocity.

Discussion

This study demonstrates a high prevalence of ejection murmurs among mature boxer dogs, and boxers with murmurs are more likely to have higher aortic velocities. In addition, auscultation was a sensitive screening test for the identification of dogs in this population with an aortic velocity > 2.0 m/s. For most dogs, the observed changes in murmur grade and aortic velocity on a one-year follow up evaluation were not clinically significant.

Over 50% of healthy mature boxers in this study demonstrated a low-grade (1-3/6) ejection murmur at the left heart base. The high prevalence of ejection murmurs found in this population parallels previous reports describing European boxer dogs.[1-3] In most circumstances, such murmurs reflect either obstruction to ventricular outflow (aortic or pulmonic stenosis) or presumed physiologic flow disturbances that are unrelated to a cardiac abnormality (functional murmur). Aortic stenosis is well-recognized in the boxer breed, with odds ratios of relative risk up to 8.6.[5,6] Physiologic murmurs are also thought to be common among boxers and may result from an exaggerated response to sympathetic tone or an anatomic variant in the LVOT not related to stenosis, such as aortic hypoplasia.[1,3] Although the true etiology of ejection murmurs in the boxer breed is unknown, their presence presents a clinical diagnostic dilemma.

Nevertheless, these data document that the presence of a basilar ejection murmur is useful in predicting aortic velocity in boxers. This is clinically

important because aortic velocity currently represents the non-invasive “gold standard” for identification of dogs with mild AS. In general, dogs with ejection murmurs had higher aortic velocities than dogs without murmurs (respective medians of 2.11 and 1.72 m/s), and a strong positive correlation was detected between murmur grade and peak aortic velocity. Auscultation was a sensitive screening test for the identification of dogs with an aortic velocity over 2.0 m/s (sensitivity of 86.9%), although the specificity of auscultation (65.8%) was lower. These findings validate the common practice of using auscultation to screen dogs for high velocity blood flow prior to further evaluation with Doppler echocardiography, a more specific but expensive test.

In the clinical situation, mature dogs that demonstrate an ejection murmur on examination typically undergo echocardiography to distinguish a pathologic murmur from a physiologic murmur. The latter is associated with a normal echocardiogram, while dogs with congenital heart defects demonstrate changes in Doppler blood flow velocities, color flow disturbances, and usually 2-D abnormalities.[20] Provided an imaging lesion in the LVOT accompanies an elevation in the aortic velocity, the diagnosis of AS is straightforward. However in the absence of a detectable abnormality on 2-D echocardiography, the diagnosis relies on measurement of the peak aortic velocity.

In this study, we recorded peak aortic velocity from the subcostal echocardiographic imaging plane. Although a reference range for normal subcostal aortic velocities in dogs has not been established, this position permits the most parallel imaging to aortic blood flow and consequently yields

the most accurate aortic velocities.[15] We evaluated our results using a 2.0 m/s cut off point, which is a commonly reported upper limit for normal subcostal aortic velocity.[19] Other investigators consider velocities up to 2.25 m/s within the normal range.[21]

It is difficult to distinguish normal vs. abnormal aortic velocity in the absence of a normal subcostal reference range. Nevertheless, an increase in aortic velocity is a major determinant in the diagnosis of AS, and hence the distinction is important for a breed predisposed to this congenital defect. Even though dogs with mild AS do not typically suffer sudden death or congestive heart failure, they do show an increased risk of developing endocarditis.[6] More importantly, the genetic basis of AS is established in Newfoundland dogs[11] and is presumed in other breeds as well. Therefore, the correct classification of dogs likely has substantial breeding implications. Assuming the diagnostic criteria for AS is based upon the presence of an ejection murmur with an aortic velocity > 2.0 m/s, 36% of the boxers in this study would have been diagnosed with AS. Seventeen percent would still fit the criteria even if the aortic velocity cut-off point were increased to 2.25 m/s.

Alternatively, the high prevalence of left basilar ejection murmurs and 'mildly elevated' aortic velocities in boxer dogs may represent physiologic changes that are unrelated to any anatomic cardiac defect. The influence of sympathetic tone on these parameters has been recognized.[1,7,24] Dynamic changes in the ventricular loading conditions associated with sinus arrhythmia, such as increased preload and/or decreased afterload with longer R to R

intervals, may explain some intermittent murmurs and highly variable aortic velocities.[16] Furthermore, 'normal' aortic velocity for the boxer dog may be different from the 'normal' velocity of other breeds. In this study, dogs with murmurs were more frequently younger and male. In addition, male dogs showed a trend toward higher aortic velocities. It may be that young male boxers more commonly experience the physiologic changes that cause functional murmurs and mild increases in aortic velocity.

Most dogs did not show a clinically significant change in auscultatory or Doppler aortic velocity findings on a one-year follow up evaluation. However, the second examination revealed a new murmur in 4 dogs (8%) and failed to reproduce a previously identified murmur in 10 dogs (21%). Intermittent murmurs in boxers have been reported,[1] and this may explain the apparent discrepancy. Another potential explanation is that murmurs were present at both examinations but were not detected because of excessive respiratory noise or excitability of the dog. Six dogs (13%) showed a decrease in aortic velocity from > 2.0 m/s to $\leq$ 2.0 m/s; they tended to be older ($p = 0.082$). For an additional 8 dogs (17%), the aortic velocity increased from $\leq$ 2.0 m/s to > 2.0 m/s; this change may be clinically significant. The average increase for those dogs was 0.31 m/s, and the largest individual change was from 1.84 m/s to 2.43 m/s. The variability in murmur grade and aortic velocity between visits may represent structural changes in the LVOT over time,[13] or it may simply reflect different physiologic conditions at separate examinations, such as level of excitement, stage of estrus cycle, or time waited for evaluation.

The relationship between aortic velocity and grade of ejection murmur must be evaluated in light of the fact that auscultation and Doppler examination are not conducted simultaneously. Perceived inconsistencies between these variables may be related to dynamic (minute to minute) changes. Such labile characteristics of ventricular outflow may explain the absence of a murmur in 11 dogs with an aortic velocity over 2.0 m/s. For the repeated evaluations in this study, it may also explain the relative wide variation of change in aortic velocity even as the murmur grade remained unchanged.

While this study considers a relatively large number of boxer dogs, there are a number of limitations. Anatomic evidence for LVOT obstruction was assessed by only one standard echocardiographic plane, the right parasternal long axis view. It is possible that some of the boxers might have had identifiable 2-D abnormalities in complementary echocardiographic planes. In addition, pulmonic stenosis was not systematically excluded by Doppler studies as a cause for the basilar ejection murmur in each dog. Pulmonic stenosis is recognized in the boxer breed; however, the reported prevalence is much less than that of AS. In a recent survey of 500 boxers in Italy, 89 (18%) were identified with congenital heart disease. Isolated pulmonic stenosis was diagnosed in only 9 dogs (2%), while 57 (11%) had subvalvular AS and 22 (4%) had both AS and pulmonic stenosis.[3] A confounding limitation was our inability to control or quantify variations in autonomic tone that may impact the development or grade of a cardiac murmur and the measured aortic velocity.

Despite these limitations, this study demonstrates a high prevalence of both ejection murmurs and equivocally increased aortic velocities among a large group of healthy North American boxer dogs. Whether these findings represent a variant of normal or signify the presence of a genuine cardiac malformation remains debatable, and further comparative echocardiographic, pathologic and genetic studies are necessary to address this clinical question.

Footnotes

[a] GE Medical, Horten, Norway

[b] Echopac, GE Vingmed Ultrasound, Horten, Norway

[c] Sigma Stat for Windows version 2.03, San Rafael, CA

[d] Gaber CE. Normal pulsed Doppler flow velocities in adult dogs, in *Proceedings*. 5th ACVIM Forum 1987; 923.

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CHAPTER 3

ECHOCARDIOGRAPHIC ASSESSMENT OF THE LEFT VENTRICULAR OUTFLOW TRACT

Introduction

Aortic stenosis (AS) is one of the most common canine congenital heart defects.[1-3] Dogs with severe stenosis demonstrate a prominent left basilar ejection murmur, a marked elevation in the aortic velocity, and a structural lesion within the left ventricular outflow tract (LVOT) making the clinical diagnosis straightforward. In contrast, mildly affected dogs generally have softer murmurs with a smaller elevation in aortic velocity, and overt structural lesions are less commonly seen.[4;5] In healthy dogs, aortic velocity is the primary determinant of murmur presence and intensity. The two main factors influencing aortic velocity include the minimal LVOT area and the left ventricular stroke volume.[6-8] Accordingly, stenosis or hypoplasia of the LVOT may cause an increased velocity. However, findings compatible with mild aortic stenosis may also be observed in the setting of altered cardiohemic dynamics, independent of a structural defect. Thus, factors that increase stroke volume (SV), such as increased preload, decreased afterload, or increased inotropy, may increase aortic velocity independent of LVOT area.[8;9]

In the boxer dog, soft left basilar ejection murmurs and mild elevations in aortic velocity suggestive of mild AS are common. Previous reports have observed typical murmurs in 50-80% of adult boxers,[10;11] and the results of chapter 2 identified the combination of a murmur and elevated aortic velocity in 36% of 201 healthy adult boxers. The genesis of these findings is uncertain, and differentiating between murmurs associated with physiologic changes that alter SV and those associated with AS is challenging. Clear echocardiographic parameters that distinguish dogs with mild aortic stenosis from those with a morphologically normal LVOT and a physiological murmur are lacking. This distinction is particularly important given the heritable nature of AS in some breeds, and the risk of apparently normal or mildly affected dogs producing more severely affected offspring.[4] We hypothesized that boxer dogs with murmurs have smaller LVOT dimensions but similar SV estimates when compared to boxers without murmurs and non-boxer dogs without murmurs. The objective of this study was to non-invasively investigate the LVOT size and to estimate SV in boxers with and without cardiac murmurs suggestive of AS, and compare these results to those obtained in a control group of age and size matched non-boxer dogs without heart murmurs.

Materials and Methods

Subjects

Adult boxer dogs between the ages of one and five years were recruited for possible participation. All dogs were auscultated by two of the investigators, and 30 apparently healthy boxers were identified and evaluated prospectively. Boxers were evaluated and selected to create for two equal- sized groups: Group I-boxers with a soft, left-basilar ejection murmur (grade 1-3/6), and Group II-a control group of boxers without detectable murmurs. An additional, 15 apparently healthy one to five year old non-boxer dogs without detectable murmurs were also recruited as a second control group for evaluation (Group III). All dogs weighed between 15 and 45 kg. Age, weight, body condition score, gender, and current medications were recorded. Exclusion criteria included thyroid supplementation, medication with a cardiovascular drug, or females either pregnant or within one week of estrus at the time of examination.

Echocardiography

An echocardiogram was performed on each dog without sedation by a single observer (SLK) blinded to the results of auscultation. Echocardiography was performed on a Vingmed System V[a] with a continuous electrocardiogram using a 5 MHz transducer (2D, M-mode study) and a 2.5 MHz transducer (Doppler study). Dogs were positioned in right and left lateral recumbency, and images were collected from the dependent hemi-thorax. Subcostal examination was conducted in the right lateral position. A single observer (SLK) performed all echocardiographic measurements off-line using saved digital cineloops.

Examinations were coded in the echocardiograph system using a random numbers table, such that measurements were made blinded to the results of physical examination, breed, and name of the dog

M-Mode echocardiogram

The echocardiographic examination was conducted using standard techniques with dogs in right lateral recumbency. The left ventricle was imaged from the right parasternal position at the level of the papillary muscles to obtain a short axis tomogram. M-mode measurements of the left ventricular diastolic diameter (LVDd) and left ventricular systolic diameter (LVDs) were obtained, and fractional shortening was calculated as (LVDd - LVDs)/LVDd.[12]

2-D Echocardiogram of the LVOT

The 2-D examination of the LVOT was performed from the right parasternal long and short axis planes and from the left parasternal (cranial) long axis view of the aorta. The subvalvular, valvular, and supravalvular regions of the aorta were scrutinized subjectively for evidence of a narrowing or abnormal morphology with each view.

In order to compare LVOT dimensions across groups of dogs, a minimum of 6 cardiac cycles were stored for each of the following image planes. Measured diameters and areas were recorded from 2 to 6 high quality images for each of 3 phases within the cardiac cycle: end-diastole (at the onset of the R wave); mid-systole (immediately following opening of the aortic valve); and end-systole (at the end of the T wave). Although the diameter of the LVOT was expected to change over the cardiac cycle, it was not clear which phase

would be most valid for analysis. Thus, dimensions from the three measured phases of the cardiac cycle were averaged prior to statistical analysis.

The smallest diameter of the immediate subvalvular region was measured from the right parasternal long axis view (parallel to the valve annulus; Figure 3.1).

The diameter of the aortic annulus was measured between the hinge points of the aortic leaflets from the right and left parasternal long axis views (Figures 3.1 and 3.2). The annulus area was determined by planimetry of the internal border from the right parasternal short axis view at a level where all three leaflets could be seen (Figure 3.3). Aortic valve (AV) excursion was measured from the right and left long axis imaging planes as the distance between the opened aortic valve leaflets (mid-systolic time point only, Figure 3.2).

Diameters from two anatomic sites in the supravalvular region were measured from the left parasternal view: the sinotubular (ST) junction (the most proximal portion of the ascending aorta just distal to the sinuses of Valsalva) and at a location ½ of the width of the ST junction distal to the ST measurement (ascending aorta; Figure 3.2). The area of the supravalvular region was measured by planimetry of the internal borders of the aorta using the right parasternal short axis view (at a level where the aortic valve leaflets are no longer imaged; Figure 3.4).

Doppler Echocardiogram (LVOT)

Color flow Doppler was used to determine the presence or absence of aortic insufficiency and systolic turbulence within the LVOT using the right parasternal long axis, right parasternal short axis, left apical 5-chamber, and left parasternal imaging planes. Doppler settings (Nyquist limit, pulse-repetition frequency, variance, gate size, turbulence map, baseline, and power) were standardized and remained unchanged during and between echocardiograms.

Spectral Doppler examination of the LVOT was conducted from the subcostal and left apical 5-chamber views. For each velocity recording, the cursor was aligned parallel to flow using concurrent 2-D and color-flow Doppler echocardiography. Optimal signal strength was obtained by observing the real time spectral display and by listening to the audio signal of the Doppler system. Left ventricular outflow tract spectral signals were obtained from the subcostal view using continuous wave Doppler at two time points during the study: as soon as the dog was positioned on the echocardiograph table in right lateral recumbency (baseline) and following completion of the right-sided examination, when the dog is was more accustomed to the procedure and typically more relaxed (mid-study). From the left apical view, the LVOT was interrogated with pulsed-wave Doppler for evidence of sudden acceleration of the outflow tract velocity. Velocity spectral recordings were made at four locations within the LVOT: at a point just distal to the aortic leaflet tips in systole (ascending aorta), at the aortic valve (AV) annulus, within the left ventricle adjacent to the midpoint of the anterior (septal) mitral valve leaflet in diastole (subvalvular), and at the

start of the LVOT adjacent to the tip of the anterior mitral leaflet in diastole (LV; Figure 3.5). If a specific sampling site could not be recorded with pulsed-wave Doppler due to aliasing of the spectral signal, high pulse repetition frequency (HPRF) Doppler was activated with the most distal sample volume placed at the region of interest. A minimum of six high quality velocity spectra were recorded at each site. The outer envelope of each velocity signal was traced, and the peak velocity, mean velocity, and stroke distance (velocity time integral, VTI) were recorded. The instantaneous heart rate corresponding to each spectral signal was derived from the preceding R-R interval.

Right heart echocardiogram

In order to calculate effective orifice area, the diameter of the pulmonary valve annulus and pulmonary artery spectral Doppler (pulsed wave) were measured from the right and left parasternal short axis imaging planes. Two to six measurements were made in a manner similar to the LVOT diameter and Doppler signals.

Coefficient of Variation

Coefficient of variation (CV) was calculated for the measured LVOT variables, including LVOT diameters, areas, and spectral analysis. Repeated measurement of the same beat/image was performed 4 to 6 times on the same day and by the same observer. Mean CV for each of the diameters and areas was determined from a minimum of 4 different images and from a minimum of 2 dogs. Mean pulsed-wave Doppler CV was calculated from repeated measurement of 12 different spectral signals from 3 dogs, and mean

continuous-wave Doppler CV was calculated from 5 different spectral signals from 2 dogs.

In order to assess overall variability in peak LVOT velocity and heart rate, the interbeat, intraobserver CV was determined at each Doppler location using each measured spectral signal from all dogs.

Calculations

Dimensions and hemodynamic estimates were indexed to body surface area. Indexed outflow tract area, stroke volume index (SVI), and effective orifice area index (EOAI) were calculated (Table 3.1).[7;13] Left ventricular SVI was calculated at two different locations: at the AV annulus (with the calculated area from an average of the annular diameters and the pulsed-wave stroke distance at the aortic valve annulus) and at the ascending aorta (using the calculated area from the ST diameter with the ascending aorta pulsed-wave stroke distance). Right ventricular SVI was also calculated in 2 different ways: using the calculated area from the pulmonary annulus diameter and the pulmonary artery stroke distance from the right parasternal imaging views only as previously published [14] and using the averaged diameters and stroke distances from the left and right parasternal imaging views.

In order to identify acceleration of flow within the LVOT, numeric differences in adjacent pulsed-wave peak velocities were calculated. Ratios of adjacent diameters and areas were also calculated to identify an abrupt change in LVOT dimension.

Statistics

Data reported in the text represent the mean +/- standard deviation, unless otherwise stated. Differences in continuous variables between the groups were assessed using analysis of variance. When significant differences were found, Tukey's post-test was performed. Diameters, areas, velocity, stroke distance, heart rate, dimension ratios, adjacent velocity differences, and Doppler CV were analyzed with two-way repeated measures ANOVA, using group designation and location or timing as factors. Differences within individual factors (not including the interaction between factors) are reported in the text as 'pooled' data. For instance, as part of two-way repeated measures ANOVA statistical test, all diameters from different LVOT locations are 'pooled' to evaluate group differences before the differences between groups for each location are assessed.

Relationships between LVOT size and velocity/stroke distance and between heart rate and velocity/stroke distance were assessed using Pearson's correlation coefficient.

Calculated and measured areas were evaluated for the ability to predict subcostal and ascending aortic velocity using multiple linear regression.

Statistical calculations were performed by a computer software program,[b] and an alpha error of 0.05 was chosen as statistically significant for all analyses.

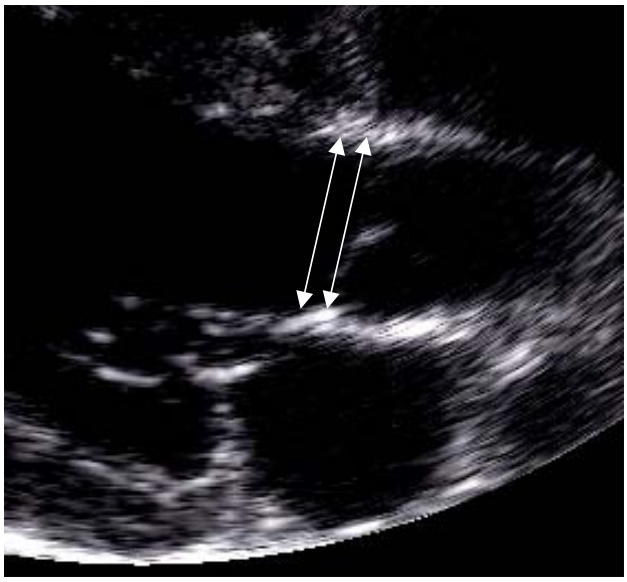

Figure 3.1: Right parasternal long axis with measured subvalvular and valvular annulus diameters.

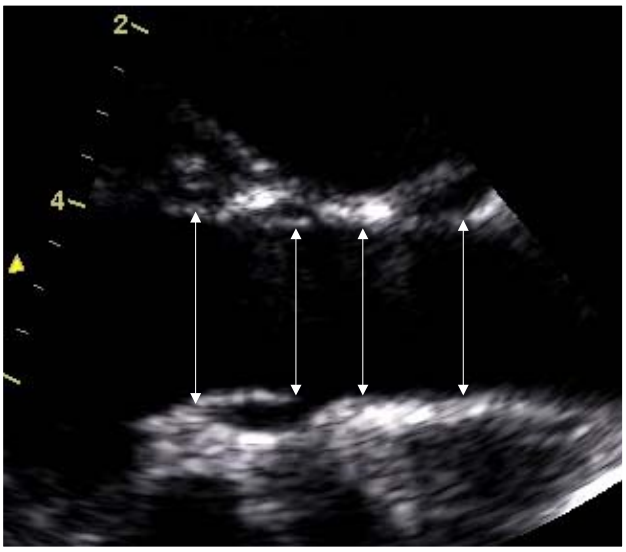

Figure 3.2: Left parasternal long axis with measured valve annulus, aortic valve excursion, sinotubular junction, and ascending aorta diameters.

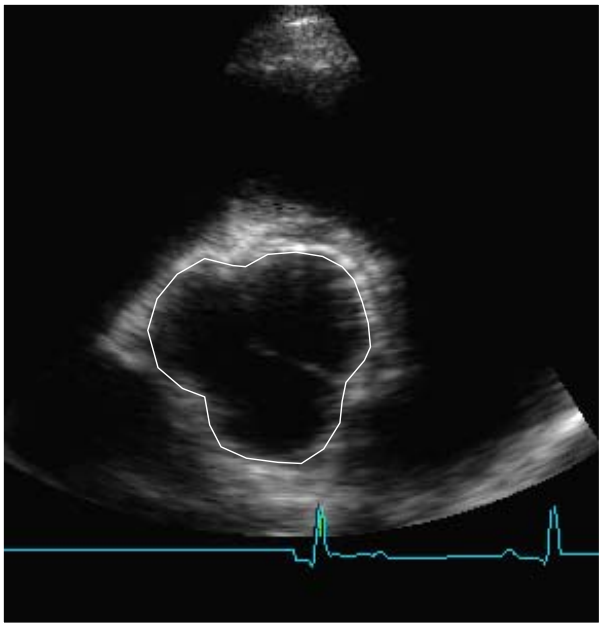

Figure 3.3: Right parasternal short axis with measured valve annulus area

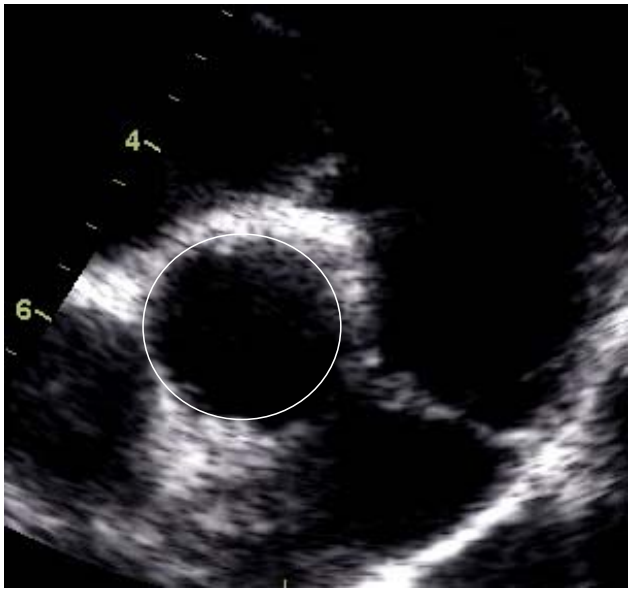

Figure 3.4: Right parasternal short axis with measured supravalvular area

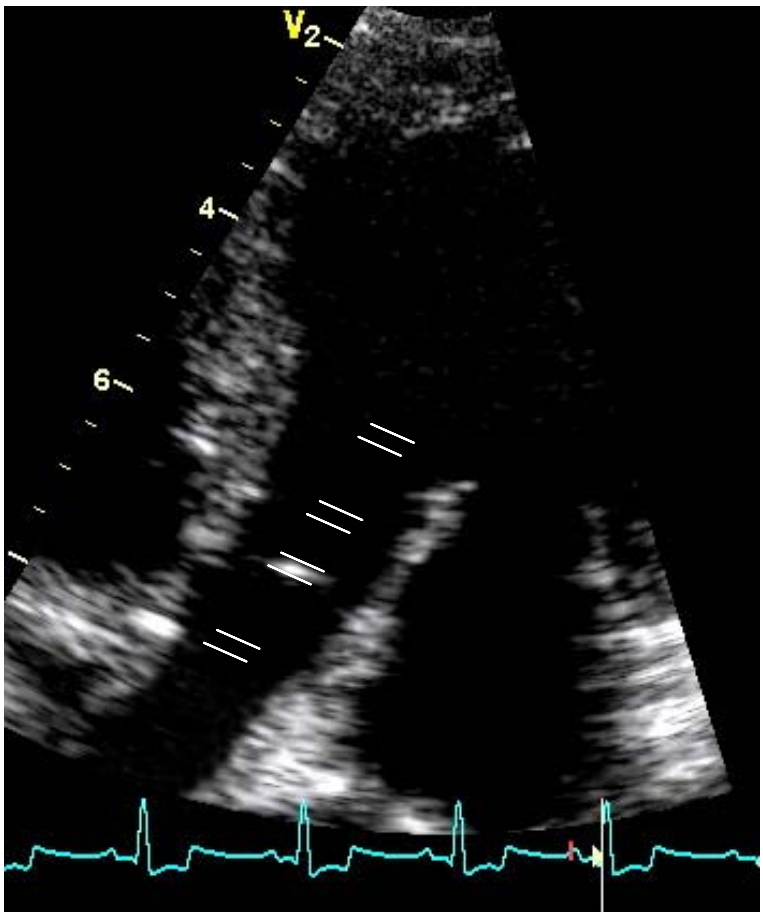

Figure 3.5: Left apical view with LVOT locations of pulsed-wave interrogation.

Indexed Area	= π (diameter/2)^2 / BSA
Stroke Volume Index (SVI)	= Area x stroke distance / BSA
EOAI	= (Area $_{PA}$ x stroke distance $_{PA}$ /stroke distance $_{SC}$)/BSA

BSA = body surface area; PA = pulmonary artery; SC = subcostal

Table 3.1: Calculations for echocardiographic data.

Results

Group characteristics

Baseline characteristics between groups were similar. No differences in body weight, body condition score, age, or baseline heart rate were found. Mean body weights for groups I (boxers with murmurs), II (boxers without murmurs), and III (non-boxers) were 27.5 kg +/- 5.7, 27.5 kg +/- 5, and 29.3 kg +/- 6.5, respectively. Median body condition score for all groups was 3/5. Mean age for groups I, II, and III were 2.8 years +/- 1.4, 2.4 years +/- 1.3, and 3 years +/- 1.2, respectively. Baseline heart rates (during physical examination) for groups I, II, and III were 111 bpm +/- 22, 117 bpm +/- 20, and 118 bpm +/- 29, respectively. Group I included 10 females (3 neutered) and 5 males (2 neutered). There were 9 females (1 neutered) and 6 males (1 neutered) in group II, and group III included 6 neutered females and 9 males (8 neutered).

M-Mode echocardiography

Each dog had a fractional shortening greater than 25%. Fractional shortening was not different between the groups (group I = 35% +/- 5, group II = 32.5% +/- 4.8, group III = 32.8% +/- 5.2), although the power for this test was very low (0.064). Likewise, there were no differences in the indexed LVIDd between groups (group I = 4.4 cm/m^2 +/- 0.65, group II = 4.3 cm/m^2 +/- 0.7, group III = 4.2 cm/m^2 +/- 0.6, power = 0.05).

2-D echocardiography

Only three dogs (one from group I, one from group II, and one from group III) had an overt 2-D lesion of the LVOT. In each case, the lesion was described as mild thickening of the aortic valve leaflets.

Diameter

For all dogs, the pooled valve annulus (left parasternal view, not accounting for group distinction) was larger than all other measured diameters within the LVOT, and the AV excursion and ST junction diameters were the smallest (Figure 3.6). Pooled average LVOT diameters were larger for group III than for group I ($p=0.049$; not accounting for differences in LVOT location). At individual locations, group III diameters remained larger than group I at the ST junction ($p = 0.002$) and ascending aorta ($p= 0.02$; Figure 3.6). No differences were found between groups I and II for any of the measured LVOT diameters ($p>0.19$). Within group I, the annular and subvalvular diameters were larger than the AV excursion, ST junction, and ascending aorta diameters, and the ascending aorta was larger than the ST junction diameter (Table 3.2). For group II dogs, the valve annulus (left parasternal view) was larger than all other diameters. Among group III dogs, individual LVOT diameters were not different ($p = 0.09 – 1$; Table 3.2).

Area

Pooled average areas (not accounting for location) were larger for group III than for groups I and II ($p <0.001$; Figure 3.7). Pooled annulus area was larger than supravalvular area ($p<0.001$; not accounting for the group

distinction). However, individual locations were not statistically different between or within groups (Table 3.2). Effective orifice area index was larger for group III dogs than for group I dogs ($p = 0.03$; group I = 2.17 cm^2/m^2 +/-0.55, group II = 2.43 cm^2/m^2 +/-0.5, group III = 2.76 cm^2/m^2 +/-0.58).

Calculated diameter and area ratios of adjacent locations within the LVOT were not different between groups of dogs ($p=0.5$).

Doppler echocardiography

Color flow Doppler

Aortic regurgitation was found in dogs from each of the 3 groups: 3 group I dogs, 2 group II dogs, and 1 group III dog. Subjective evidence of LVOT turbulence (by color flow) was recorded for groups I, II, and III at the subvalvular region in 2, 2, and 4 dogs, respectively; at the valvular region in 12, 7, and 6 dogs, respectively; and at the supravalvular region in 2, 1, and 1 dog, respectively. No dog had evidence of mitral valve regurgitation.

LVOT velocity and stroke distance

Pooled average LVOT velocity and stroke distance (not accounting for location) were higher for group I dogs than for group II and III dogs ($p<0.006$), and group II dogs had higher pooled velocity and stroke distance than group III dogs ($p<0.05$).

Subcostal velocity and stroke distance were highest among group I dogs ($p<0.001$), although there was no difference between groups II and III for these variables ($p>0.2$; Figure 3.11). All groups were statistically different from one another at the ascending aorta location ($p <0.04$). At this location, group I

velocity and stroke distance were greater than groups II and III, and group II was greater than group III. Valve annular velocities and stroke distance were higher among group I and II dogs compared with group III ($p<0.004$), although groups I and II were not different from each other at this location (velocity $p=0.3$, stroke distance $p=0.063$). Subvalvular and LV velocities and stroke distances were not statistically different between groups of dogs ($p>0.27$). No differences were found between baseline and mid-study subcostal velocities or stroke distance ($p>0.9$). Thus, these values were averaged for subsequent analysis.

Comparing within groups of dogs, subcostal velocities were statistically greater than ascending aorta velocities for group III ($p=0.03$), but this difference was not found in groups I and II ($p>0.12$; Figures 3.8-3.10). Subcostal stroke distance was not different from the ascending aorta stroke distance for any group ($p>0.3$). Ascending aorta velocity was higher than the velocity at the valve annulus for group I and group III dogs ($p<0.05$), but not for group II ($p=0.5$). Ascending aorta stroke distance was higher than annular stroke distance only for group I ($p = 0.011$). For groups I and II only, annular velocity and stroke distance were higher than subvalvular measurements ($p<0.001$), and subvalvular velocities and stroke distance were higher than LV measurements ($p<0.007$).

Pooled average differences in adjacent LVOT velocities (velocity step-up, not accounting for location) were larger for group I dogs than for group III dogs ($p = 0.04$; group I = 0.45 m/s, group II = 0.28 m/s, group III = 0.24 m/s,

Figures 3.8-3.10). There were no statistical differences between locations, although the power for this test was low (0.2).

Stroke volume index was numerically higher in group I dogs compared to groups II and III for all methods of calculation (Table 3.3). Differences between groups reached statistical significance only between groups I and III for the left ventricular SV index at the AV annulus ($p=0.004$). On average, group I dogs ejected 4 to 7.3 ml/m^2 more blood per beat than group II dogs (Table 3.3).

Heart rate (based on R-to-R interval) was not different throughout the echocardiogram with one exception ($p=0.07$-1). The heart rate during the baseline subcostal spectral Doppler recording was significantly higher than the heart rate during the mid-study subcostal recording ($p=0.001$; 108 vs 98 bpm, respectively). For all groups, the pooled mean heart rate during the echocardiogram was 100 bpm.

Subcostal and ascending aortic velocities and stroke distance indices were negatively correlated with indexed valvular and supravalvular areas ($p<0.003$; $r^2 = 0.21 – 0.38$). Subcostal and ascending aortic velocities were also weakly and negatively correlated with the indexed ST junction diameter ($p<0.03$; $r^2 = 0.12$), whereas subcostal and ascending aortic stroke distance indices were weakly and positively correlated with the indexed subvalvular diameter ($p<0.04$, $r^2 = 0.11 – 0.14$) and annular diameter (left, $p<0.05$, $r^2 = 0.09 – 0.11$). For all measured spectral signals, ascending aortic and subcostal stroke distance and ascending aortic velocity were weakly and negatively correlated with heart rate ($p<0.001$, $r^2 = 0.06 – 0.15$).

The calculated area from the ST junction diameter statistically predicted the ascending aortic pulsed-wave velocity based on multiple linear regression ($p = 0.003$, coefficient = -0.665), however none of the measured or calculated areas were able to predict the subcostal velocity ($p>0.2$).

Right heart echocardiogram

Indexed pulmonary annulus diameters and peak pulmonic velocities were not different between groups of dogs. Mean indexed diameters for groups I, II, and III were 2.09 cm/m^2 +/- 0.16, 2.05 cm/m^2 +/- 0.29, and 2.09 cm/m^2 +/- 0.19, respectively. Mean velocities were 1.22 m/s +/- 0.14, 1.13 m/s +/- 0.15, and 1.11 m/s +/- 0.21, respectively. Pulmonic ejection velocities were < 1.5 m/s in all dogs. Pulmonic stroke distance indices were not statistically different between groups ($p = 0.06$, power = 0.38). Mean stroke distance indices for groups I, II, and III were 19.4 cm/m^2 +/- 3.5, 17.2 cm/m^2 +/- 2.3, and 16.5 cm/m^2 +/- 3.6, respectively.

Coefficient of Variation

Mean intrabeat, intraobserver CV ranged from 1.56% to 3.55% for diameter variables and was 3.24% to 3.85% for the area variables. Mean intrabeat intraobserver CV for the pulsed and continuous wave Doppler variables were 0.89% to 1.05% (peak velocity), 3.12% to 3.38% (mean velocity), 2.2% to 2.4% (stroke distance), and 0.44% to 0.59% (heart rate).

Mean interbeat, intraobserver CV for the peak velocity was statistically higher for group I dogs (7.3% +/- 3.4) than for groups II and III (5.3% +/- 2.9 and 5.1% +/-2.3, respectively; $p<0.004$). The mean interbeat, intraobserver CV for heart rate was statistically higher in group I dogs (20%) compared with groups II and III (11.2% and 9.5%, respectively; $p=0.001$).

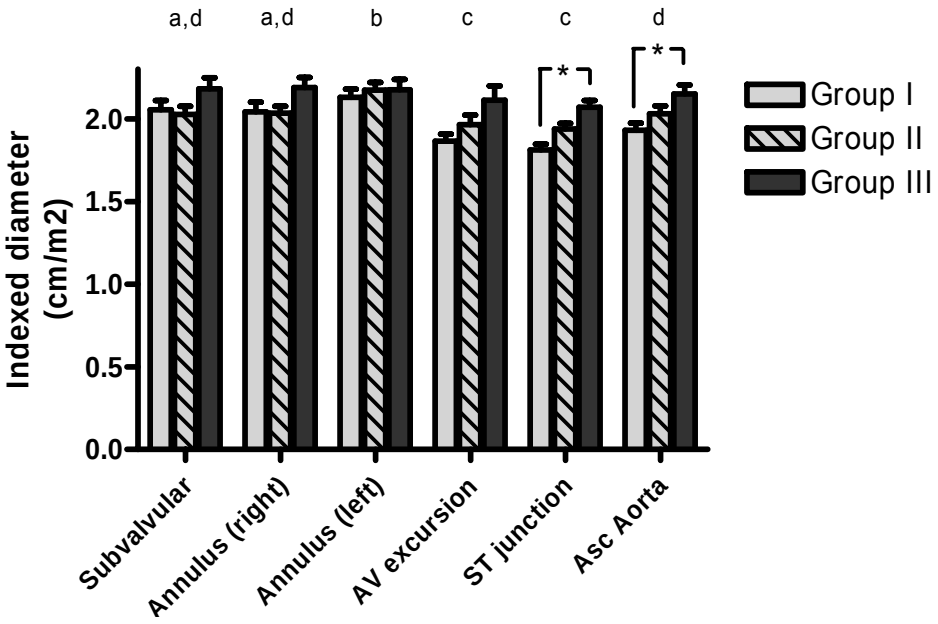

Figure 3.6: Mean LVOT diameters.

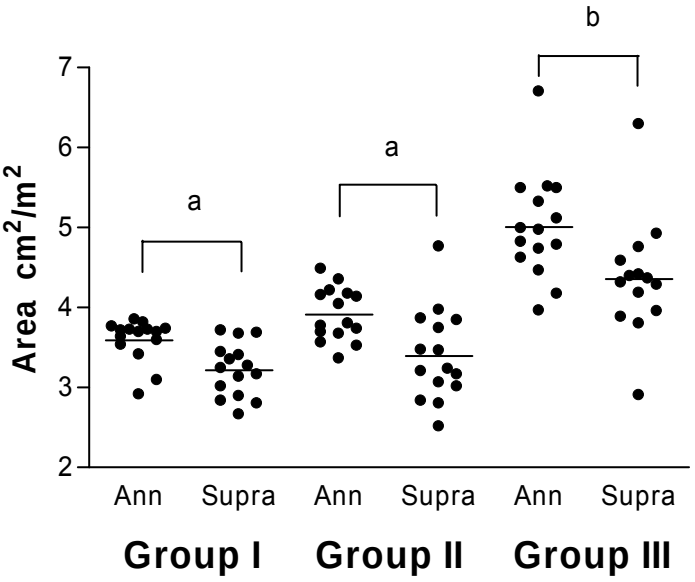

Figure 3.7: Measured LVOT areas.

	Indexed Mean **Diameter** (SEM) cm/m^2						Indexed Mean **Area** (SEM) cm^2/ m^2	
Group	Subvalvular	Annulus (right)	Annulus (left)	AV Excursion	ST Junction	Ascending Aorta	Annulus Area	Supra-Valvular Area
I	2.06 [a] (0.03)	2.04 [a] (0.03)	2.13 [a] (0.03)	1.85 [b,c] (0.04)	1.81 [b] (0.03)	1.93 [c] (0.03)	3.59 (0.08)	3.22 (0.08)
II	2.03 [d] (0.03)	2.03 [d] (0.03)	2.17 [e] (0.03)	1.96 [d] (0.03)	1.94 [d] (0.03)	2.03 [d] (0.03)	3.91 (0.08)	3.39 (0.08)
III	2.16 (0.03)	2.17 (0.03)	2.18 (0.03)	2.05 (0.04)	2.07 (0.03)	2.13 (0.03)	4.98 (0.08)	4.36 (0.08)

Table 3.2: Mean LVOT diameters and areas (SEM) and within group differences.

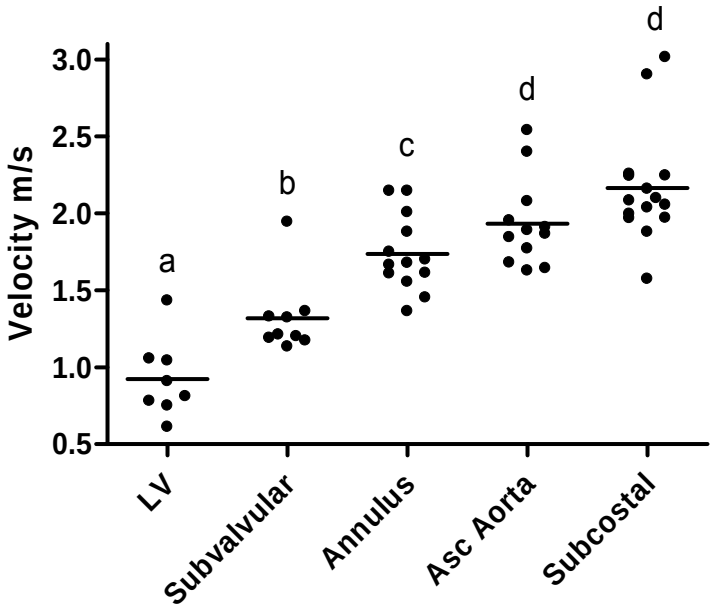

Figure 3.8: LVOT velocities for group I dogs.

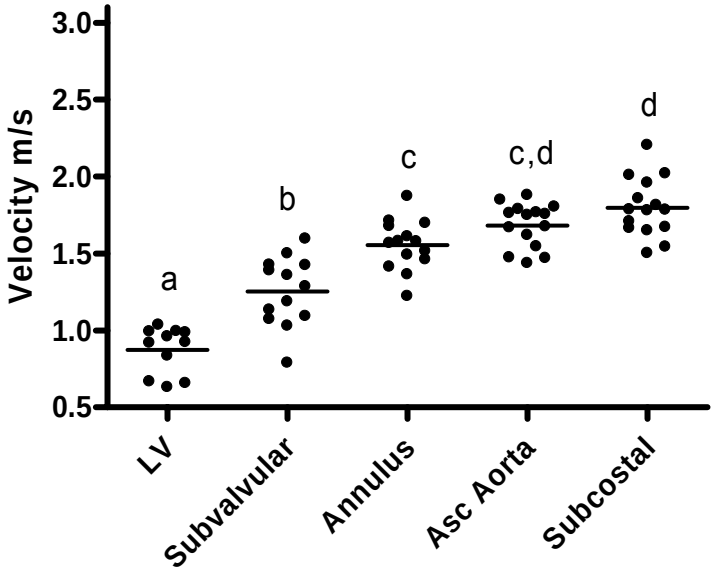

Figure 3.9: LVOT velocities for group II dogs.

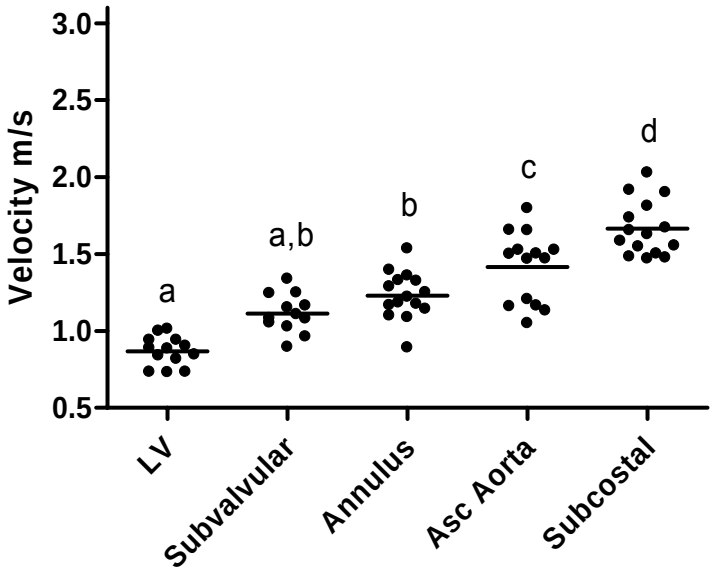

Figure 3.10: LVOT velocities for group III dogs.

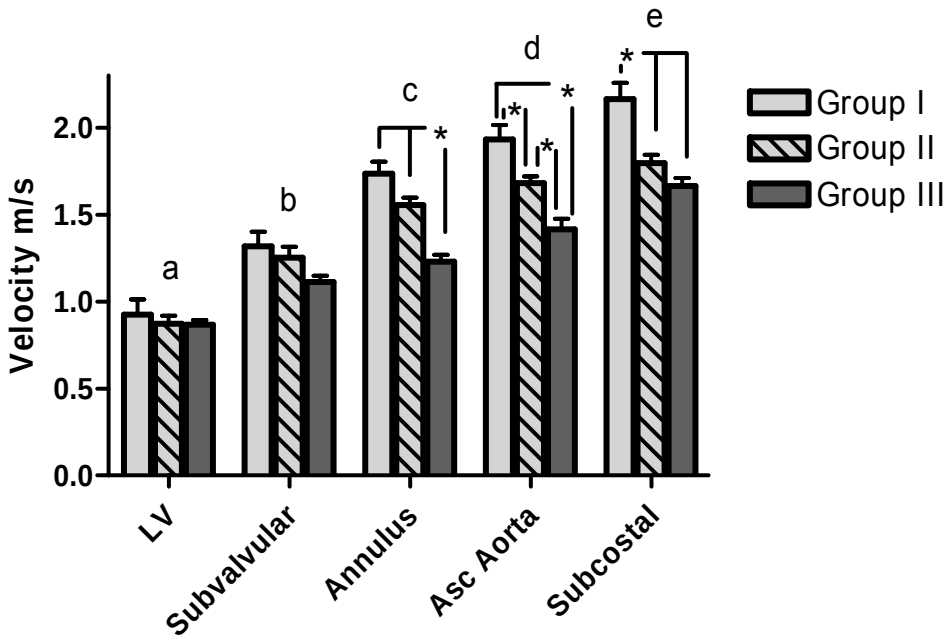

Figure 3.11: Mean (SEM) LVOT velocities for all groups across each LVOT location.

	Group I (ml/m^2)	Group II (ml/m^2)	Group III (ml/m^2)	Group I minus Group II (ml/m^2)	P value (I vs. II)
Left ventricular SVI At AV Annulus Mean (SEM)	63.3 (2.4)	56 (2.8)	51 (2.1)	7.3	0.12
Left ventricular SVI Asc Aorta Mean (SEM)	54.1 (2.7)	50.1 (1.9)	51.4 (2.3)	4	0.45
Right ventricular SVI (Right echo views only) Mean (SEM)	54.4 (3.2)	47.7 (2.3)	49.7 (2.9)	6.7	0.24
Right ventricular SVI (ave right & left echo) Median	53.8	46.7	52	7.1	0.07

Table 3.3: Stroke volume index across groups of dogs.

Discussion

The results of this study indicate that boxer dogs have smaller LVOT area indices and higher LVOT velocities than weight-matched non-boxer dogs, independent of “aortic stenosis”. The cause of soft ejection murmurs and elevations in aortic velocity among some boxer dogs remains uncertain, as significant differences in LVOT structure and/or flow were not detected between boxers with murmurs and those without murmurs. However, the trend for boxers with murmurs to have a higher stroke volume suggests that flow may play a prominent role.

Structural differences within the LVOT were found only between boxers with murmurs and non-boxer controls, not between the two boxers groups. Subjective evidence supporting aortic stenosis was uncommon in this study, and the incidence of mild aortic valve thickening was equally distributed among controls and boxers with murmurs. Objectively, boxers with murmurs had statistically smaller sinotubular junctions, ascending aortas, and effective orifice areas, when compared with non-boxer dogs. In contrast, all area and diameter measurements of the LVOT, as well as the effective orifice area calculation, were not different between boxers with murmurs and boxer controls. These findings may suggest that a structural lesion/narrowing within the LVOT is not responsible for murmur generation in boxer dogs. Alternatively, it is possible that techniques employed in this study were not sensitive enough to detect the structural difference between boxer controls and boxers with murmurs. Although not statistically significant, mean diameters, areas, and EOA index

were numerically smaller in boxers with murmurs compared with both control groups, and these values were numerically (not statistically) smaller in boxer controls compared with non-boxer controls (Figures 3.6 and 3.7, Table 3.2). It is possible that with a greater number of dogs evaluated and higher statistical power, statistically significant structural differences would have been evident between the groups of boxers.

In contrast to the 2-D echocardiogram, the Doppler examination identified statistical differences between the boxers with murmurs and both control groups. Left ventricular outflow tract velocities and stroke distance conducted from the subcostal imaging plane and at the ascending aorta location were higher in boxers with murmurs (Figure 3.11). This finding was expected and is consistent with chapter 2 and prior reports.[15] When all three groups were compared, the stroke volume index was not different between groups. However, there was a trend for boxers with murmurs to eject 13% more blood per beat than the boxer controls. With a greater number of dogs studied, it is possible that this numerical difference would be more convincing.

In the diagnosis of aortic stenosis, some reports discuss finding an abnormal, abrupt increase in flow velocity, or a velocity step-up.[16,17] We looked for this by calculating differences in velocity from adjacent locations within the LVOT. Boxers with murmurs had a statistically larger overall step-up across the LVOT than non-boxer controls only. Although the mean numeric difference between boxers with murmurs and boxer controls was not statistically

significant (0.45 m/s vs. 0.28 m/s, respectively), this may represent a clinically important difference between groups. In people, a velocity step-up over 0.3 m/s is considered abnormal, even in high flow states.[18] In this study, mean differences for all groups of dogs were greater than 0.3 m/s at the start of the LVOT. The mean difference in boxer controls was equivalent to this cut-off point at the mid-LVOT, and mean differences in boxers with murmurs were consistently above this value (0.46 m/s, 0.39 m/s, and 0.49 m/s from the LV to the ascending aorta, respectively; Figure 3.8). Hence, boxers with murmurs showed a consistently larger increase in velocity rather than an abrupt step-up across the LVOT. This finding does not support the presence of a discrete stenosis within the LVOT, wherein an abrupt acceleration of flow would be expected.[17] Rather, it may be more consistent with either a high flow state, multiple LVOT stenoses, or diffuse, relative hypoplasia of the LVOT.

Other echocardiographic evidence that would support a diagnosis of aortic stenosis includes abnormalities in color flow, including the presence of systolic turbulence and/or aortic insufficiency. Aortic insufficiency has been reported in 87% of dogs with aortic stenosis.[c] This finding is rare in the normal population and considered a highly sensitive marker of aortic stenosis.[16] However, in this study trivial or mild aortic insufficiency was identified in 3 boxers with murmurs and 3 dogs without murmurs (2 boxers). It is possible that the two control boxers actually had aortic stenosis or that aortic insufficiency is more common in normal dogs that is generally recognized.[4] Interestingly, the boxer control dog with valve thickening was not the same dog that

demonstrated valve insufficiency. Systolic turbulence (spectral dispersion) within the LVOT is also an expected finding with aortic stenosis.[16,19] However, the interpretation of the color flow mapping is subjective and largely dependent upon the Doppler settings of the echocardiograph. In addition, acceleration of laminar flow and a poor signal to noise ratio can lead to a false positive finding of 'turbulence'.[20] Such factors may explain the very high prevalence of LVOT 'turbulence' (>50%) found among all groups of dogs in this study.

The effect of heart rate on aortic velocity was also assessed in this study. Aortic velocity was negatively, though weakly, correlated with heart rate, likely reflecting an effect of variable preload. Overall, there was no significant difference in the heart rate during the echocardiogram, with the exception of a lower heart rate at the mid-study subcostal velocity time point when compared with that measured at the start of the study (baseline). This finding supports the clinical impression that dogs were more relaxed in the middle of the study. Interestingly, there was no statistical difference in the averaged subcostal velocities between those time points, although the range in differences for individual dogs was from 0 to 0.38 m/s. Boxers with murmurs demonstrated a more variable heart rate, likely reflecting a greater tendency for sinus arrhythmia. In addition, this group showed greater variation in aortic velocity, which supports the effect of heart rate on this parameter.

This study was designed to incorporate 2 groups of control dogs, boxers without murmurs and non-boxer dogs without murmurs. Comparisons between these two groups of control dogs underscore the importance of reference

values for each breed of dog. For instance, mean ascending aortic velocity for boxers without murmurs was 0.25 m/s higher than non-boxer dogs in this study (Figure 3.11). The average velocity at this location for non-boxers (1.42 m/s +/- 0.23) is similar to previously published reference values,[6,14] and the average velocity for boxers without murmurs (1.68 m/s +/- 0.14) is similar to previous published values for this breed of dog.[21] Additionally, the pooled measured area was statistically smaller in boxer controls than for non-boxers. Although numerical differences in LVOT size did not reach statistical significance at individual area locations, mean indexed LVOT annular and supravalvular areas were approximately 1 cm^2/m^2 smaller in the boxer control dogs compared with the non-boxer controls (Figure 3.7, Table 3.2). It is likely that this numerical difference is clinically important, and may be consistent with a prior observation of aortic hypoplasia in boxers,[21] potentially independent of murmur status.

Some of the findings in this study support the presence of a structural difference in the LVOT as the cause of the soft ejection murmur and increased aortic velocities commonly found in boxer dogs. A negative correlation between LVOT velocity and LVOT area was observed, and ST junction diameter helped predict the ascending aortic velocity. Additionally, LVOT diameters suggested a more heterogeneous nature of the LVOT in boxers with murmurs compared with both control groups, in which the ST junction was statistically smaller than all other diameters. Among boxer and non-boxer control dogs, the ST junction was similar to most of the other diameters (Table 3.2).

However, hemodynamic factors may still play a role in determining ejection velocity and development of murmurs in boxers. The low correlation coefficients relating LVOT area to velocity suggest that less than 40% of the change in velocity or stroke distance may be explained by a change in LVOT area. In addition, boxers with murmurs tended to have a higher SVI. Crude estimates of preload (LVIDd) and LV contractility (fractional shortening) were not different between the groups of dogs, although the statistical powers were very low (<0.1). Importantly, such estimates cannot fully describe changes in preload or contractility. The other important determinant of stroke volume, afterload, was not addressed in this study.

Traditional explanations of murmur generation involve the development of turbulence, created when flow exceeds the critical Reynold's number, although the presence of periodic wake fluctuations in association with shed vortices (Aeolian tones) may also play a role.[22,23,24] Factors in sound transmission to the chest wall, including chest conformation, may also help determine the presence/absence of an ejection murmur.[24] The aortic velocity varies with stroke volume and systemic vascular resistance/aortic impedance,[9] though no attempt was made to estimate or measure the latter in this study. It is possible that the smaller boxer aorta reduces the critical Reynold's number (the point at which laminar flow becomes turbulent), increasing the chance of turbulent blood flow and a subsequent murmur. This combined with a normal or slightly increased stroke volume would lead to ideal circumstances to generate a murmur and increased aortic velocity.

Limitations

There are some important limitations to the current study. The small number of dogs in each of the groups, along with the large number of measured variables, decreased the power for some of the statistical tests. This may have limited our ability to detect differences between groups, increasing the type II error. In addition, intermittent ejection murmurs have been reported in boxer dogs.[11] It is possible that some of the boxer controls (without detectable murmurs) may have had murmurs at another point in time, and this may have been an additional confounding factor adding to the lack of measurable difference between the boxers groups.

For some of the dogs, patient compliance during the echocardiogram was less than ideal. While sedation would have increased the quality of the 2-D study, it was not performed because of the adverse effect on the hemodynamic estimates of the Doppler study.[21,25] In addition, no attempt was made to measure some of the factors relevant to the etiology of murmur generation, including viscosity and ventricular afterload.

The results of this study suggest that boxer dogs (both with and without ejection murmurs) have higher outflow tract velocities and smaller left ventricular outflow tract dimensions than non-boxer dogs. This finding is relevant in the interpretation of screening dogs for mild congenital heart defects. Further studies are required to confirm the trend for boxers with murmurs to have a higher SVI and/or reduced aortic impedence than boxers without murmurs. In addition, more sensitive techniques may be employed to

investigate the presence of a structural difference within the LVOT in dogs with soft ejection murmurs, including transesophageal echocardiography, intravascular echocardiography, magnetic resonance imaging, and/or pathology.

Footnotes

[a] GE Medical, Horten, Norway

[b] Sigma Stat for Windows version 2.03, San Rafael, CA.

[c] O'Grady MR. The incidence of aortic valve insufficiency in congenital canine aortic stenosis: a Doppler echocardiographic study. *J Vet Intern Med* 1990;4:129 (abstract).

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CHAPTER 4

INVASIVE ASSESSMENT OF THE LEFT VENTRICULAR OUTFLOW TRACT

Introduction

Left basilar ejection murmurs have been described in 50 – 80 % boxer dogs.[1,2,3] In chapter 2, the combination of this type of murmur and a mild elevation in aortic velocity (2 to 3 m/s) was present in 34 % of 201 healthy boxers. Although such findings may be consistent with aortic or subvalvular aortic stenosis, echocardiographic 2-D lesions confirming the presence of stenosis are uncommonly detected.[4,5] Murmurs and elevated aortic velocities typical of mild aortic stenosis may also be observed with physiologic changes in cardiohemic dynamics, independent of a cardiac defect.[6] For these reasons, a reliable diagnosis for the presence or absence of congenital heart disease remains uncertain in many boxers, even following detailed 2-D and Doppler echocardiography. Nevertheless, aortic velocity and the calculated pressure gradient (PG) are major diagnostic criteria.

One of the important determinants of aortic velocity is the minimal left ventricular outflow tract (LVOT) area. Aortic hypoplasia (including a smaller indexed aortic annulus and sinotubular junction) has been previously suggested in boxer dogs, although the data supporting these findings are not available

in the literature.[1,7,8] In individual boxers with mildly elevated aortic velocity, findings of convincing stenosis are uncommon. In chapter 3, only one of 15 boxers with ejection murmurs demonstrated an imaging lesion compatible with LVOT obstruction (aortic stenosis). Surprisingly, such lesions were identified with an equal frequency between the groups (one boxer with an ejection murmur, one boxer without a murmur, and one non-boxer without a murmur). In that study, the indexed LVOT area was smaller in boxers compared with non-boxer dogs, while no difference in LVOT size was found between those boxers with ejection murmurs and those without murmurs. This finding suggested the boxer LVOT may be different from non-boxer dogs, however it did not help explain why some boxers have murmurs (and elevated aortic velocities) while others do not.

The other major determinant of aortic velocity is stroke volume. Determinants of stroke volume include preload, afterload, and contractility, wherein an increased preload, decreased afterload, or increased contractility may all increase stroke volume. Echocardiographic data from chapter 3 suggested that boxers with murmurs tend to have a higher stroke volume index (SVI) when compared to boxers without murmurs. This finding suggested an important role of stroke volume in generating the ejection murmurs and elevated aortic velocities in boxer dogs, and it prompted the current study.

Prior to the advent of echocardiography, angiography was the gold-standard test in the diagnosis of morphologic cardiac lesions, while the measured PG from catheterization of adjacent cardiac chambers was

considered the gold-standard test in the diagnosis and assessment of severity for stenotic lesions. Two-dimensional and Doppler echocardiography has since become the standard test in the clinical cardiology laboratory. Compared with angiography, current ultrasound technology may be superior at detecting subtle morphologic lesions. Prior studies have shown excellent correlation between catheterization gradients and Doppler-derived gradients in dogs with severe aortic stenosis ($r = 0.99$).[9] Doppler gradients are estimated from the simplified Bernoulli equation: $PG = 4 (velocity)^2$.[10] Similar agreement has been presumed in all severities of LVOT obstruction, although this has not been specifically investigated. It is possible that assumptions in the simplified Bernoulli calculation may not be valid for trivial or mild stenosis.

The major objective of this study was to evaluate the role volume infusion (increased preload), and sympathetic tone have on the LVOT hemodynamics in boxer dogs. Secondary objectives were to compare non-invasive and invasive measures of PG and LVOT dimension. In addition, the angiogram was reviewed for evidence of imaging lesions in boxers with equivocal or mild aortic stenosis.

We hypothesized that boxers would show an exaggerated increase in PG, SVI, and cardiac index (CI) in response to sympathetic stimulation (as with dobutamine infusion), and that boxer and control dogs would show an equivalent increase in response to volume infusion (following an osmotic agent). In addition, we hypothesized that Doppler-derived PG would predict catheter-derived PG and that echocardiographic LVOT dimensions would

predict angiographic dimensions, each with good agreement. Furthermore, we hypothesized that imaging lesions would not be seen on the angiogram in boxers with equivocal or mild aortic stenosis.

Materials and Methods

Subjects

This study was conducted in accordance with, and under the guidelines of the Animal Care and Use Committee of the Ohio State University College of Veterinary Medicine. Written consent authorizing study participation was obtained from each client providing a dog.

Eight apparently healthy adult boxers dogs were recruited for possible participation from a pool of boxers with previously recorded soft left basilar ejection murmurs and subcostal aortic velocities between 2.0 and 3.0 m/s. Six mature, weight-matched Hound dogs were purchased to serve as a control group. Age, weight, body condition score, gender, and current medications were recorded. Exclusion criteria included thyroid supplementation, medication with a cardiovascular drug, or females either pregnant or within one week of estrus at the time of examination.

Baseline Echocardiography

An echocardiogram was performed on each dog without sedation using a Vivid VII [a] with a continuous electrocardiogram and a 3.5 MHz transducer. Dogs were positioned in right and left lateral recumbency, and images were

collected from the dependent hemi-thorax. Subcostal examination was conducted in the right lateral position. A single observer (SLK) performed all echocardiographic measurements off-line using saved digital cineloops.

2-D Echocardiogram of the LVOT

The 2-D examination of the LVOT was performed from the right parasternal long and short axis planes and from the left parasternal (cranial) long axis view of the aorta. The subvalvular, valvular, and supravalvular regions of the aorta were scrutinized subjectively for evidence of a narrowing or abnormal morphology with each view. This subjective assessment was determined by a consensus from 2 of the investigators (SLK and KMM).

In order to compare echocardiographic LVOT dimensions with angiographic dimensions, a minimum of 6 cardiac cycles were stored for each of the following image planes. Measured diameters were recorded from 2 to 6 high quality images for each of 3 phases within the cardiac cycle: end-diastole (at the onset of the R wave); mid-systole (immediately following opening of the aortic valve); and end-systole (at the end of the T wave). Although the diameter of the LVOT was expected to change over the cardiac cycle, it was not clear which phase would be most valid for analysis. Thus, dimensions from the three measured phases of the cardiac cycle were averaged prior to statistical analysis.

The smallest diameter of the immediate subvalvular region was measured from the right parasternal long axis view (parallel to the valve annulus; Figure 3.1).

The diameter of the aortic valve annulus was measured between the hinge points of the aortic leaflets from the right and left parasternal long axis views (Figures 3.1 and 3.2). The diameters from each view were averaged to generate a single annular dimension.

The sinus of Valsalva was measured from the right and left parasternal long axis views across the widest portion, including 2 of the sinuses. The diameters from each view were averaged to generate a single sinus dimension.

The sinotubular (ST) junction (the most proximal portion of the ascending aorta just distal to the sinuses of Valsalva) was measured from the left parsternal long axis view (Figure 3.2).

Doppler Echocardiogram of the LVOT

Color flow Doppler was used to determine the presence or absence of aortic insufficiency using the right parasternal long axis, right parasternal short axis, left apical 5-chamber, and left parasternal long axis imaging planes. The presence or absence of mitral regurgitation was assessed from the right parasternal long axis and left apical 4-chamber imaging planes. Spectral Doppler examination of the LVOT, conducted from the subcostal view with continuous wave Doppler, was recorded at the baseline time point and at 5 additional time points during the following cardiac catheterization procedure. For each velocity recording, the cursor was aligned parallel to flow using concurrent 2-D and color-flow Doppler echocardiography. Optimal signal strength was obtained by observing the real time spectral display and by listening to the audio signal of the Doppler system. The outer envelope of at

least 5 closely-timed velocity signals were traced, and the peak and mean velocities and calculated PG were recorded. The instantaneous heart rate corresponding to each spectral signal was derived from the preceding R-R interval.

Cardiac Catheterization

Following physical examination and baseline echocardiogram, each dog underwent cardiac catheterization in order to correlate simultaneous Doppler-derived and catheter-derived PG and to compare anatomic features of the LVOT as identified by echocardiography with angiography. In addition, the effects of volume infusion and sympathetic stimulation on LVOT velocity and PG were assessed under anesthesia following iodinated contrast and dobutamine infusions, respectively.

Dogs were sedated with butorphanol (0.5 mg/kg intramuscularly). Subcostal spectral Doppler was repeated 20 minutes following sedation. Anesthesia was induced with propofol (2-4 mg/kg, intravenously to effect), and maintained with halothane (0.5-2% in oxygen, as required). Cefazolin (22 mg/kg intravenously) was administered at induction and every 2 hours until the end of the procedure. The right femoral triangle was clipped, surgically prepared, and draped. A cut-down procedure was used to obtain vascular access to the femoral artery and vein. Using a modified Seldinger technique, an 8 French introducer was placed in the femoral artery and a 7 French introducer was placed in the femoral vein.

An 8 French straight-tipped, dual micromanometer Millar catheter [b] with an end hole was advanced retrograde into the left heart over a guide wire in order to obtain high-fidelity pressure recordings from the left ventricle and ascending aorta (5 cm between micromanometers). The Millar catheter was externally calibrated in a warm saline bath. The pressure transducers were interfaced to a computerized digital signal recording system [c] equipped with two pressure amplifiers. A 7 French Swan-Ganz thermodilution catheter was advanced antegrade from the femoral vein to the pulmonary artery in order to measure cardiac output by thermodilution.

Pressure gradients

Simultaneous comparisons of catheter-derived and Doppler-derived PG (cath PG and echo PG, respectively) were determined under four different conditions: with anesthesia only, after the angiogram (simulating increased preload), and at two levels of dobutamine infusion (2.5 µg/kg/min and 5.0 µg/kg/min). In each case, dobutamine was infused for a minimum of 10 minutes before measurements were taken.

For each dog and under each condition, the average of 5-8 closely timed sinus beats was used for statistical analysis. The maximal instantaneous echo PG was calculated from the peak aortic velocity spectra by application of the simplified Bernoulli equation (PG = 4 x velocity^2),[10] while the mean echo PG was calculated by tracing the outer envelope of the spectral signal. Maximal instantaneous catheterization gradients were measured for simultaneous cardiac cycles. Only the positive early systolic gradient was considered (ie:

when left ventricular pressure was greater than aortic pressure). The instantaneous cath PG was measured as the greatest positive difference between the left ventricular and aortic pressure curves (LV pressure minus aortic pressure). The mean positive catheterization gradient was calculated by averaging all instantaneous gradients across the duration of the positive PG. The negative PG, when the aortic pressure is higher than the LV pressure during the latter half of systole, was not considered in determining any cath PG. Pressure gradient duration, time from the onset of the PG to the maximal instantaneous gradient (time to peak PG), and ejection time (from the onset of a PG to the incisura) were measured. Ratios of PG duration to ejection time and time to peak PG to PG duration were calculated. Peak to peak PG was calculated as the difference between the maximal left ventricular pressure and the maximal aortic pressure.

Cardiac output

Thermodilution cardiac output was measured [d] in triplicate at 4 time points: during anesthesia only; following contrast injection and the angiogram; and during 2.5 and 5 µg/kg/min dobutamine infusion (Dob 2.5 and Dob 5, respectively). The average cardiac output was indexed to body surface area to generate a cardiac index (CI), and the CI was divided by the heart rate to calculate stroke volume index (SVI) for each time point.

Angiogram

A left ventriculogram was performed using 2 mL/kg of iodinated contrast [e] under pressure injection at 500-600 pounds per square inch at a rate of 10

mL/sec with a linear rise of 0.5 seconds. The cine loop of the right lateral angiogram was collected and temporarily stored on the digital processing system of the fluoroscopic X-ray unit.[f] Measurements of the LVOT were made using this system. The complete angiogram was recorded on videotape, and selected images were printed on radiographic film for the permanent archive.

Measured diameters included the immediate subvalvular region, aortic valve annulus, sinus of Valsalva, ST junction and the ascending aorta (at a location approximately half of the distance between the ST junction and the brachycephalic artery). These measurements were compared with corresponding echocardiographic images, with the exception of the ascending aorta, as a similar location was not recorded echocardiographically. All dimensions were indexed to body surface area prior to statistical comparison.

Angiograms were reviewed in a blinded manner for subjective evidence of LVOT abnormalities using the permanent archives, and the results were compared with the subjective echocardiographic assessment of the LVOT.

Post-procedure all dogs were recovered from anesthesia. Boxers were returned to their owners, and controls were made available for adoption.

Statistics

Data reported in the text represent the mean +/- standard deviation, unless otherwise stated. Differences in continuous variables between the groups were assessed using analysis of variance.[g] When significant differences were found, Tukey's post-test was performed. Two-way repeated measures ANOVA was used to identify differences in continuous variables

between groups of dogs, between different time points, and between groups at individual time points.

Linear regression was used to assess the ability of Doppler-derived (echo) PG to predict catheter-derived (cath) PG and the ability of echocardiographic LVOT dimensions to predict angiographic LVOT dimensions. Bland-Altman was used to assess the agreement and bias between echo and cath PG and between echocardiographic and angiographic LVOT dimensions.

Results

Subjects

The boxer group included 3 males and 5 females, and the control group consisted of 4 males and 2 females. Mean body weights for boxers and controls were not statistically different (p=0.06; 29.4 vs. 25.3 kg, respectively). The mean age of the boxers was 5.5 years (range, 2.5 to 7 years). The ages of the control dogs were not available for analysis. The median body condition score for boxers was 3/5 (range, 2 to 3.5), and the median for controls was 2.5/5 (range, 2 to 3).

Soft left basilar ejection murmurs were identified in 8/8 boxers (two grade 1/6, three grade 2/6, and three grade 3/6) and in 4/6 control dogs (one grade 1/6, two grade 2/6, and one grade 3/6). No murmurs were detected in the other 2 control dogs.

Baseline Echocardiography

No imaging lesions within the LVOT were detected by 2-D echocardiography in 6/8 boxers and 5/6 control dogs. One boxer showed mild aortic valve thickening with restricted motion of the left coronary cusp. Lesions for the other boxer and control dog were described as mild aortic valve thickening with mild hyperechogenicity and normal leaflet excursion.

Doppler Echocardiography (baseline)

Average subcostal aortic velocities for boxers (2.46 m/s; 1.96 to 3.13 m/s) and controls (2.12 m/s; 1.77 to 2.31 m/s) were not statistically different (p=0.11). The aortic velocities for the boxers with aortic valve thickening were

2.9 and 3.13 m/s, while the velocity for the control dog with valve thickening was 1.91 m/s. Four boxer dogs demonstrated trace to 1+ aortic insufficiency, including the two dogs with morphologic lesions. Aortic insufficiency was not detected in any of the control dogs. Trace mitral regurgitation was seen in one boxer (7 years of age) and one control dog, and 1+ mitral regurgitation was seen in another control dog.

Doppler velocity and echo PG during cardiac catheterization

Aortic velocities averaged 0.14 m/s higher in boxers following sedation (range, 0.01 m/s lower to 0.34 m/s higher), and they averaged 0.19 m/s higher in control dogs (range, 0.03 m/s lower to 0.57 m/s higher). However, sedated aortic velocities and echo PG were not statistically different from baseline for both boxers and control dogs ($p>0.6$; Figure 4.1).

Aortic velocity and echo PG were statistically lower following anesthesia, compared with baseline for both groups of dogs ($p<0.003$). Individual boxer dogs showed an average 0.92 m/s velocity decrease (0.65 to 1.46 m/s), while control dogs showed an average 0.65 m/s decrease (0.42 to 0.88 m/s). This reflects an average 61% decrease in instantaneous echo PG for boxers with anesthesia and an average 51% decrease for controls (Figure 4.1).

Boxers developed higher aortic velocities and echo PG than control dogs with dobutamine (Dob 2.5 and Dob 5; $p<0.02$), while statistical differences were not detected between groups for these variables at any other time point prior to or during catheterization ($p=0.09$ to 0.18 for baseline and sedated intervals and $p=0.47$ to 0.78 for anesthesia and post-angiogram time points, Figure 4.1).

Heart rate, measured during echocardiography, was higher during Dob 5 when compared with the sedated and anesthesia time points ($p<0.032$). All other heart rates were not statistically different from one another ($p=0.053$ to 1), including the baseline and sedated heart rates ($p=0.28$).

Catheter-derived PG

The results from 3 dogs (2 controls and 1 boxer) were excluded from the catheter-derived data due to errors in calibration of the Millar catheter.

The instantaneous cath PG showed a progressive numerical increase from anesthesia to post-angio to Dob 2.5 to Dob 5 for both boxers and controls (Figure 4.2). When evaluating time point differences only, without regard to group status, the Dob 5 cath PG was statistically higher than all other time points ($p<0.012$), and Dob 2.5 was statistically higher than the anesthesia time point ($p=0.013$). These differences did not remain statistically significant when individual groups were considered ($p=0.051$). The instantaneous cath PG increased in boxers from 6.9 mmHg +/- 3.4 during anesthesia to 24.1 mmHg +/- 9.3 at Dob 5, while control dogs increased from 9.4 mmHg +/- 1.8 during anesthesia to 18.5 mmHg +/- 5.1 at Dob 5 (Figure 4.2). This represents an average 367% increase in the cath PG for boxers (64% to 639%) and an average 98% increase for controls (46% to 174%).

Pressure gradient characteristics

Time to peak instantaneous cath PG was not different between groups or time points ($p>0.29$, power = 0.05 to 0.1; pooled mean for boxers = 28 ms, for controls = 31 ms). However, the Doppler-derived time to peak PG was

statistically shorter for boxers at the post-angio, Dob 2.5, and Dob 5 time points ($p<0.04$; difference between means 10 ms), while there was no difference between the groups at the baseline ($p=0.13$), sedated ($p=0.9$), or anesthesia time points ($p=0.5$). No differences were found for the cath PG duration, catheterization-derived ejection time, ratio of cath PG duration to ejection time, or the ratio of time to peak cath PG to cath PG duration, although the power for these tests were very low (0.05 to 0.32). The positive cath PG lasted 53% of systole in the boxer dogs (13% to 71%), and it lasted 47% of systole for controls (27 to 73%).

For every dog, including boxers and controls, the positive cath PG was limited to the early portions of systole. No dog showed a positive cath PG that lasted until the incisura. In the majority of cases, the shape of the positive PG was asymmetric and early peaking as seen in Figures 4.3 to 4.5. An exaggerated gradient was seen in response to dobutamine in one dog (Figure 4.6).

Peak to peak PG

Peak to peak PG was not different between groups or time points ($p>0.3$, power <0.1; Figure 4.7). Mean peak to peak PG was < 1 mmHg for all groups and time points, with the exception of the boxer Dob 5 time point (5.3 mmHg). At the Dob 5 time point, 5 of the 7 boxers had a peak to peak PG < 5 mmHg. The highest peak to peak PG (30 mmHg) was recorded in the boxer with the lowest baseline aortic velocity (1.96 m/s) and the most dramatic increase in SVI at Dob 5 (164% increase, 79.2 ml/m^2). Subjective review of this dog's cath PG

shows an asymmetric, early peaking gradient that resumes a normal shape and relationship to the aortic pressure wave by the latter third of systole (Figure 4.6). Another boxer had a peak to peak PG of 5.6 mmHg at the Dob 5 time point (baseline aortic velocity = 2.46 m/s; SVI = 71.7 ml/m^2). The boxer with the highest baseline velocity (3.13 m/s) did not undergo the Dob 5 portion of the study due to hypothermia under anesthesia. For this dog, the Dob 2.5 peak to peak PG was 4.7 mmHg with a SVI of 85.1 ml/m^2. The boxer with the second highest velocity (2.9 m/s) was excluded from the catheter data analysis due to calibration errors. The highest peak to peak PG for an individual control dog was 2.6 mmHg (at the Dob 5 time point, SVI = 79 ml/m^2).

Cardiac index and stroke volume index

The mean cardiac index and stroke volume index showed a progressive increase from the anesthesia time point to the Dob 5 time point (Figures 4.8 and 4.9). For all dogs, the CI and SVI were statistically higher at Dob 5 compared with all other time points ($p<0.005$). Numerical differences between groups did not reach statistical significance for either CI ($p=0.09$; power = 0.65) or SVI ($p=0.08$; power = 0.35). Mean SVI for boxers and controls at the anesthesia time point were 47 ml/m^2 +/- 12 and 61 ml/m^2 +/- 5, and at Dob 5 they were 79 ml/m^2 +/- 11 for boxers and 80 ml/m^2 +/- 14 for controls (Figure 4.8). This represents an average 82% total increase in SVI for boxers (52 to 164%), compared with an average 29% increase in controls (13 to 52%, Figure 4.10). The heart rate used to calculate SVI was statistically higher at Dob 5 compared with the anesethesia and Dob 2.5 intervals ($p<0.007$).

Instantaneous PG correlations with SVI and peak to peak gradients

In the boxer dogs, instantaneous echo and cath PG were significantly correlated to both SVI and the peak to peak gradient (Table 4.1). In controls, echo and cath PG were correlated with SVI, but not to peak to peak gradients (Table 4.2).

Correlation between instantaneous echo and cath PG

The instantaneous echo PG statistically predicted the positive instantaneous cath PG with a slope of 0.81 and an intercept of 1.67 (r^2 = 0.82; Figure 4.11). Comparing the individual regression lines for boxers and controls, there was no statistical difference between the groups for the slope or the intercept (p=0.39 and p=0.11, respectively). Instantaneous echo PG overestimated instantaneous cath PG for boxers with an average bias of 2.56 mmHg +/- 4.26, while echo PG slightly underestimated cath PG for control dogs with an average bias of – 0.12 mmHg +/- 2.95 (Figure 4.11).

Angiography

Indexed angiographic diameters were significantly smaller in boxer dogs compared with controls at all measured locations within the LVOT (p<0.05), with the exception of the ascending aorta (p=0.4; Figure 4.12). The sinus of Valsalva location demonstrated the greatest difference between groups; the mean indexed boxer sinus of Valsalva was 0.56 cm/m^2 smaller than controls. At other locations that were statistically different, the indexed boxer LVOT was an average of 0.21 to 0.23 cm/ m^2 smaller. Within the control group, the sinus of Valsalva was the largest LVOT dimension (p<0.001). Within boxers, the sinus

of valsalva and the ascending aorta were the 2 largest dimensions ($p<0.001$), and they were not different from each other ($p=0.99$).

On subjective evaluation, the sinus of Valsalva for each of the boxer dogs was smaller than expected, whereas the sinuses appeared normal for each of the control dogs (figures 4.13 – 4.16). Aortic valve thickening was identified in 3 of the boxers and none of the control dogs. Baseline subcostal aortic velocities for boxers with angiographic evidence of valve thickening were 2.46, 2.46, and 3.13 m/s. The ascending aorta was dilated in 4 boxers, including 2 of those with valve thickening, and in the other 4 boxers the ascending aorta was noted to be equal to or equivocally larger than the sinuses. The dogs with dilation of the ascending aorta without angiographic valve thickening had baseline velocities of 2.1 and 2.9 m/s. In most cases, this dilatation was most prominent just proximal to the brachycephalic artery.

Correlation between echocardiographic and angiographic dimensions

Echocardiographic measurements statistically predicted the angiographic measurements with a slope of 0.73 and an intercept of – 0.5 (Figure 4.17). No statistical differences were found between the individual regression lines for different LVOT locations ($p=0.33$ for the slopes and $p=0.27$ intercepts). Echocardiographic dimensions tended to overestimate the angiographic dimensions with a mean bias of 0.22 cm/m^2 +/- 0.18 (Figure 4.17). In the figure, the four data points lying outside the 95% confidence intervals were generated from a single boxer.

Subjective morphologic comparison

Aortic valve thickening was identified by both angiography and 2-D echocardiography in the boxer with the highest baseline aortic velocity. For the boxer with the second highest baseline velocity, aortic valve thickening was identified by echocardiography, but this was not seen on angiographic review. Additionally, angiography identified valve thickening in 2 boxers for which echocardiographic lesions were not seen. Angiographic evidence of valve thickening was not seen for the control dog with this echocardiographic finding.

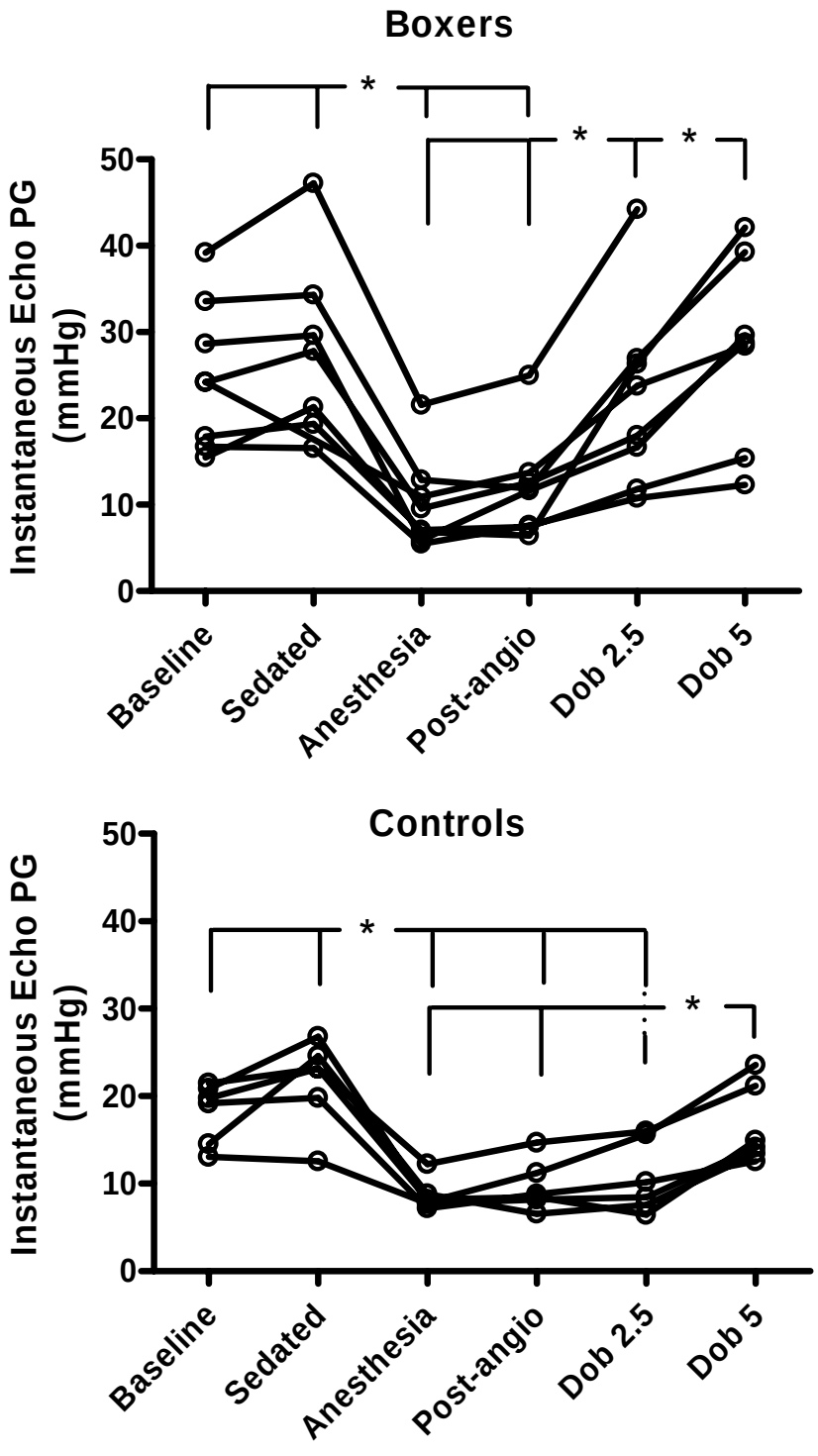

Figure 4.1: Instantaneous Echo (Doppler-derived) PG.

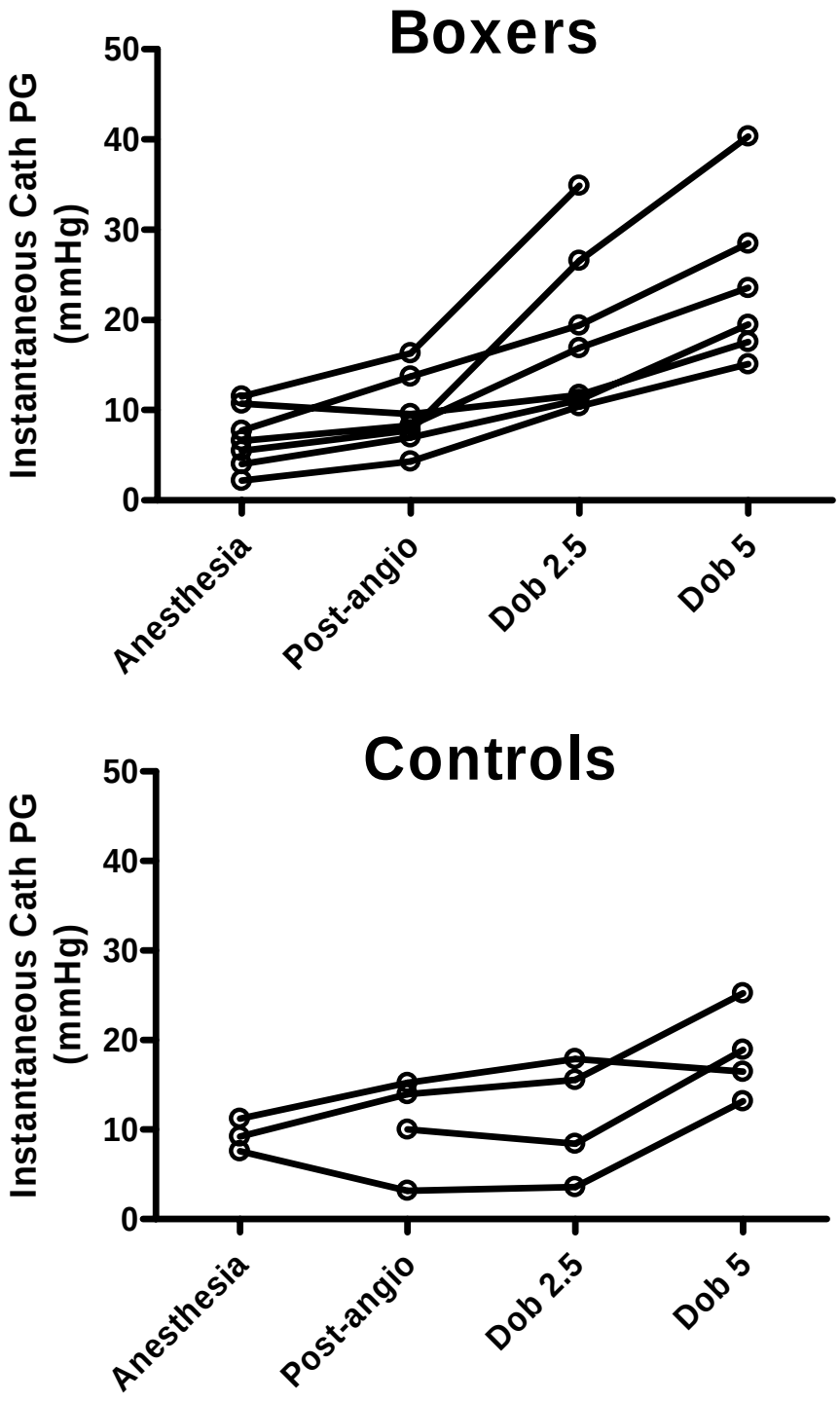

Figure 4.2: Instantaneous Cath (catheter-derived) PG.

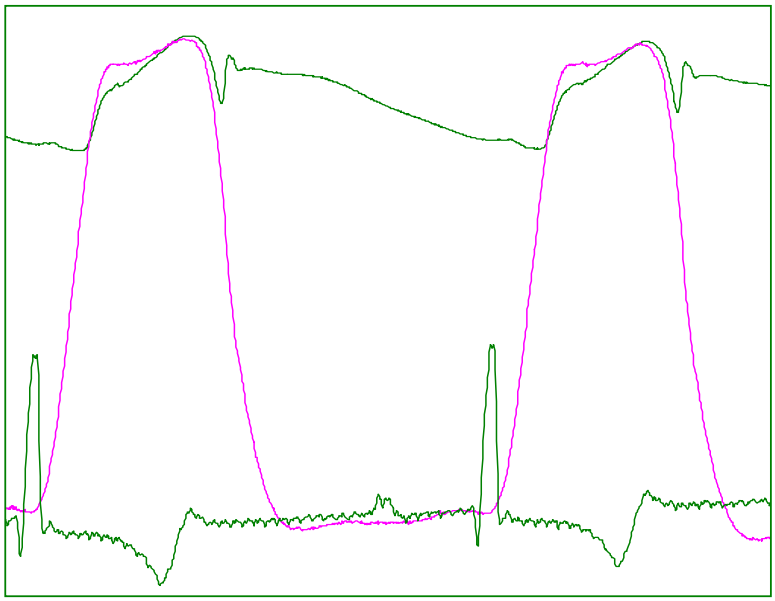

Figure 4.3: Boxer example of pressure waves, anesthesia time point.

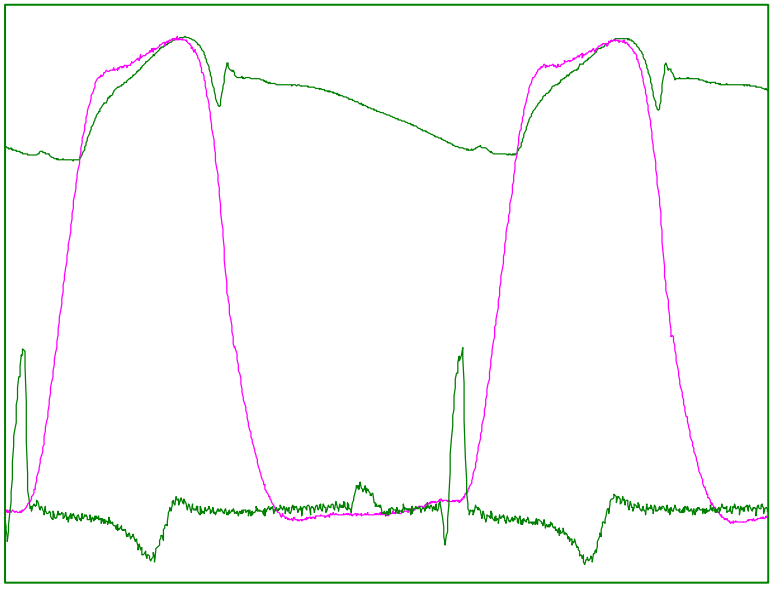

Figure 4.4: Boxer example of pressure waves, post-angiogram time point.

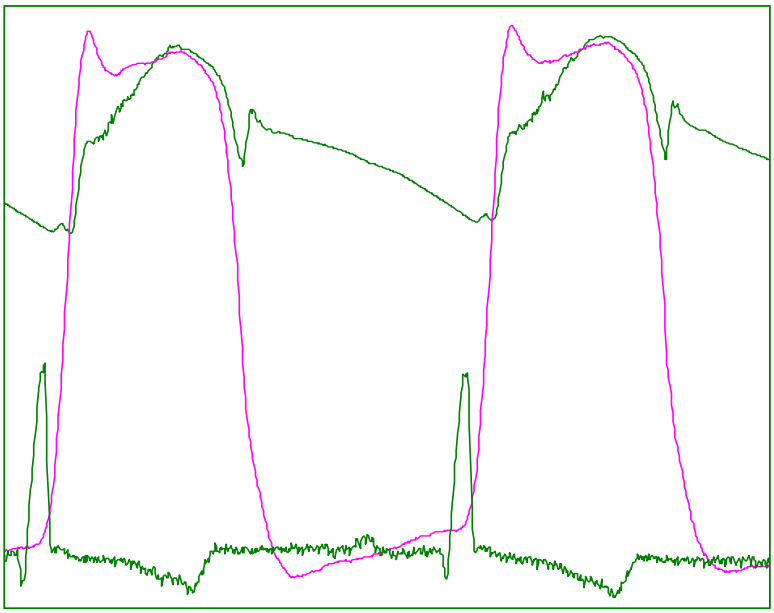

Figure 4.5: Boxer example of pressure waves, Dob 2.5 time point

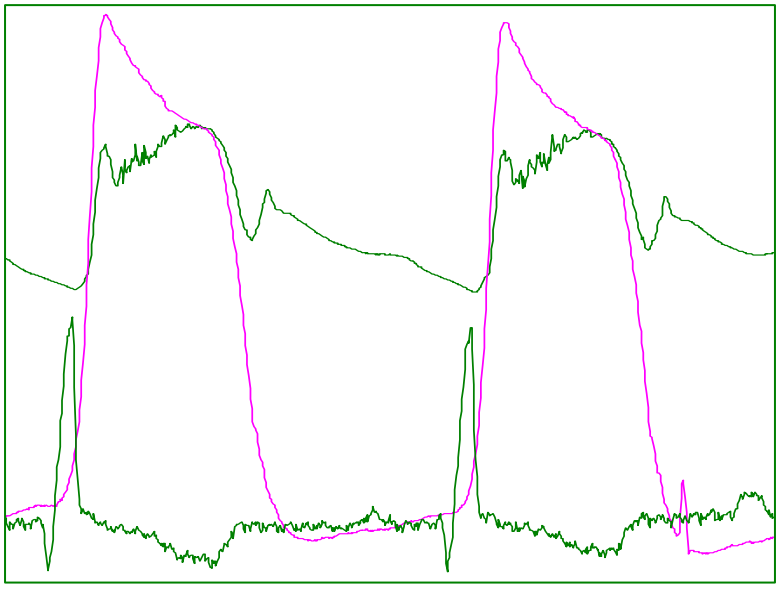

Figure 4.6: Boxer example of pressure waves, Dob 5 time point.

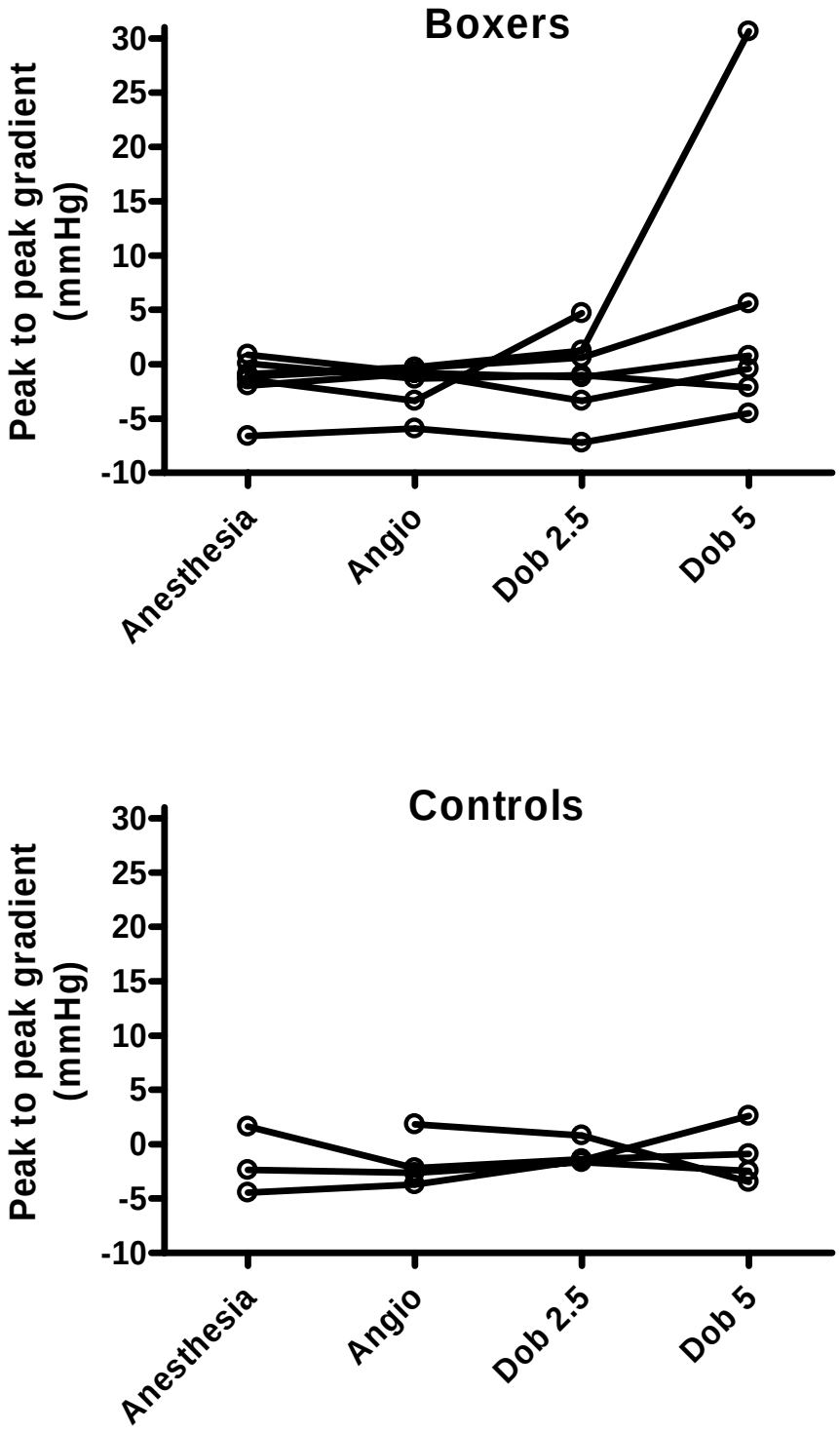

Figure 4.7: Peak to peak gradients.

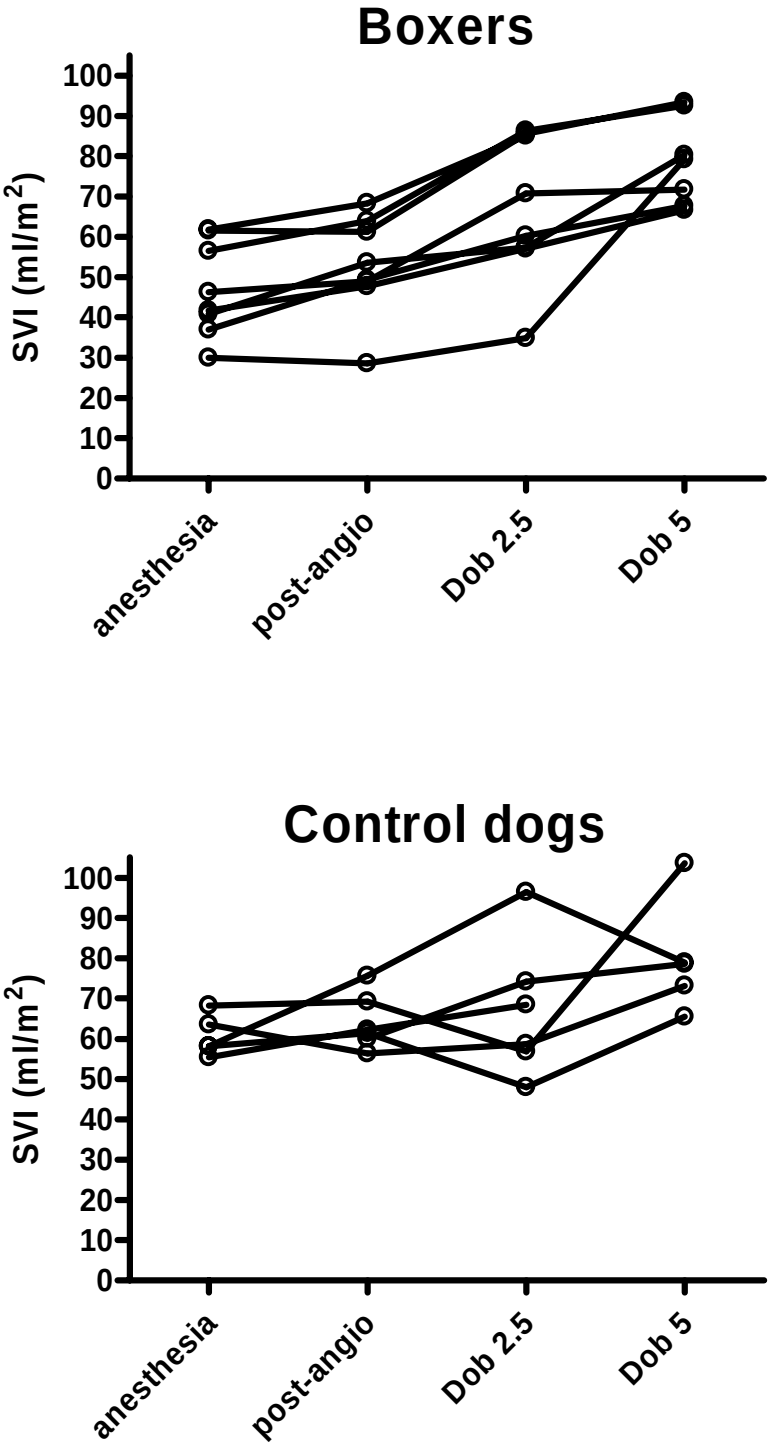

Figure 4.8: Stroke volume index for boxers and controls.

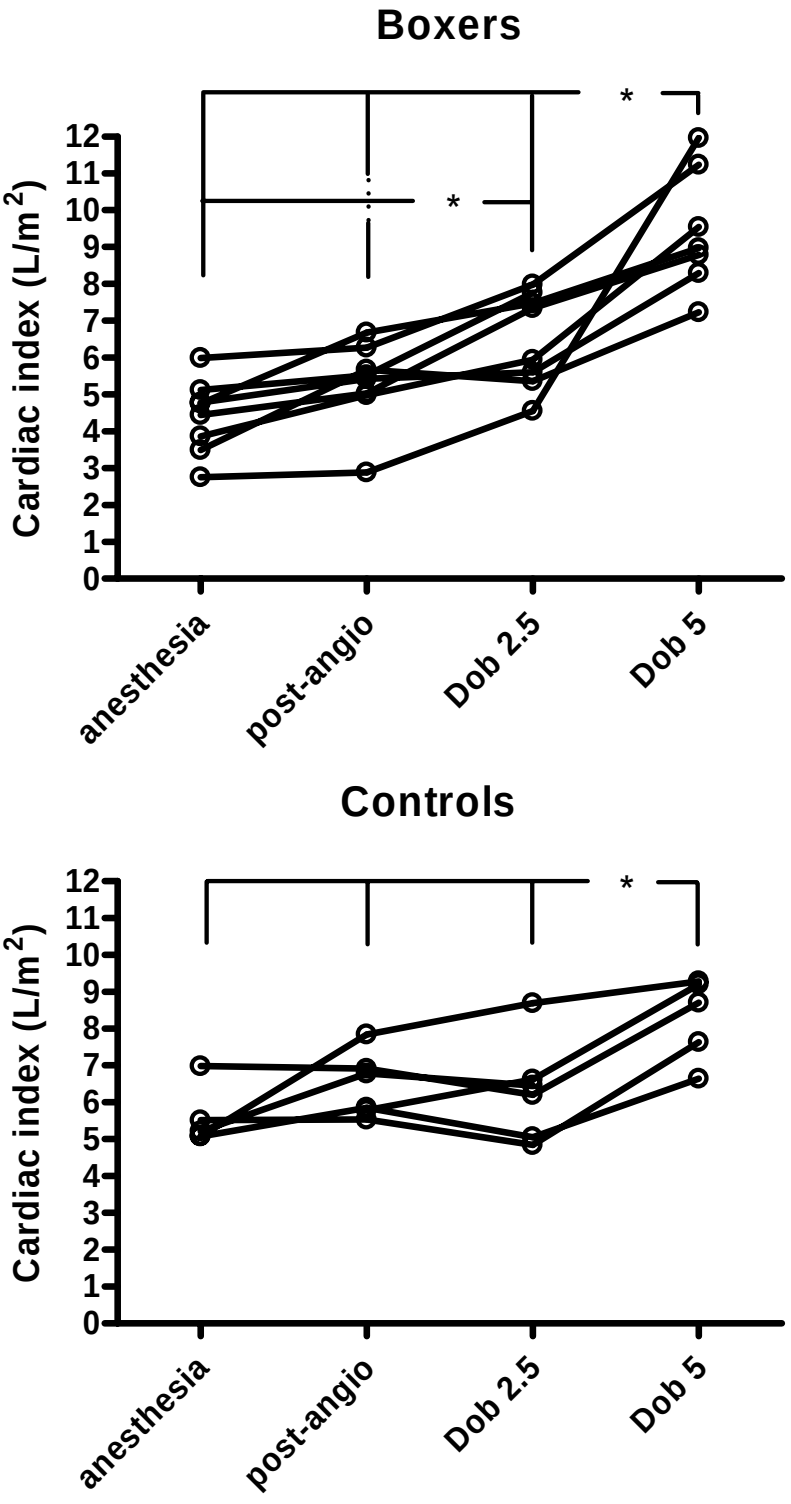

Figure 4.9: Cardiac index for boxers and controls.

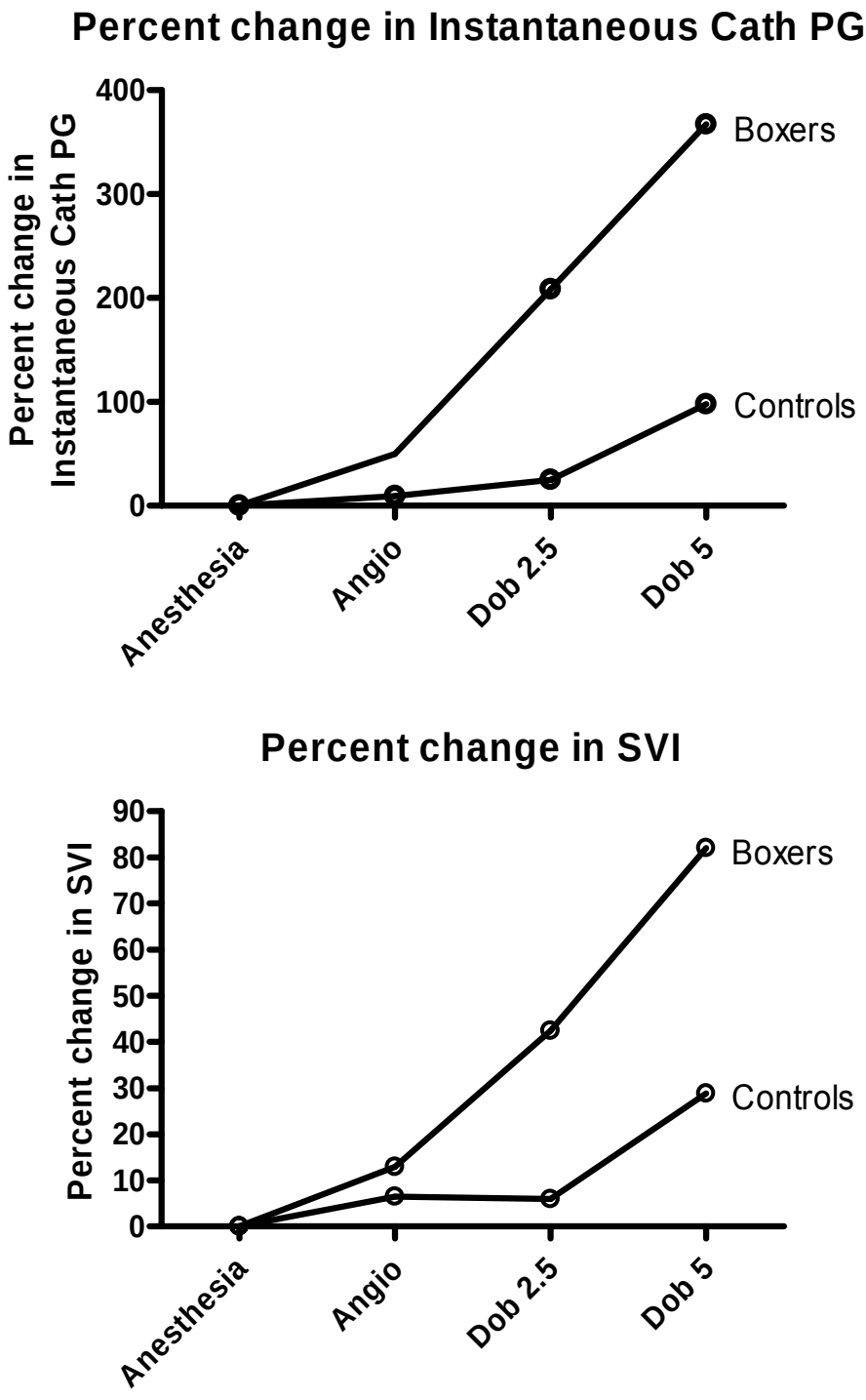

Figure 4.10: Percent change in Instantaneous Cath PG and SVI.

Boxers	Inst. Echo PG	Inst. Cath PG	Peak to Peak gradient	SVI
Inst. Echo PG	NA	0.92 *	0.62 *	0.75 *
Inst. Cath PG	NA	NA	0.68 *	0.6 *
Peak to Peak gradient	NA	NA	NA	0.28 #

* denotes p<0.001; # denotes p = 0.16

Table 4.1: Comparison of correlation coefficients for boxers.

Controls	Inst. Echo PG	Inst. Cath PG	Peak to Peak gradient	SVI
Inst. Echo PG	NA	0.87 *	0.05 #	0.59 *
Inst. Cath PG	NA	NA	0.07 #	0.74 *
Peak to Peak gradient	NA	NA	NA	-0.22 #

* denotes p <0.01; # denotes p > 0.45

Table 4.2: Comparison of correlation coefficients for controls.

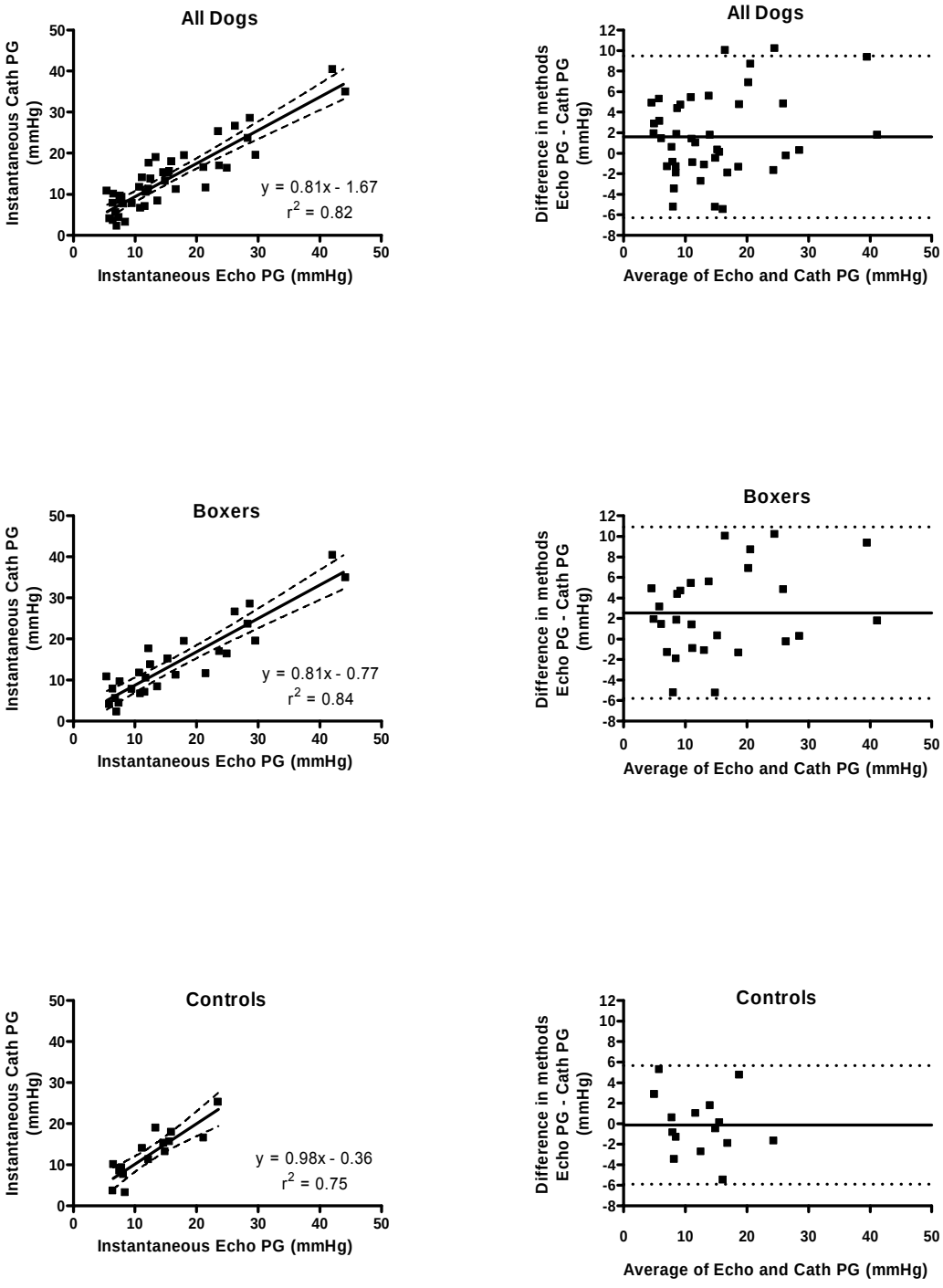

Figure 4.11: Comparison between Instantaneous Echo and Cath PG.

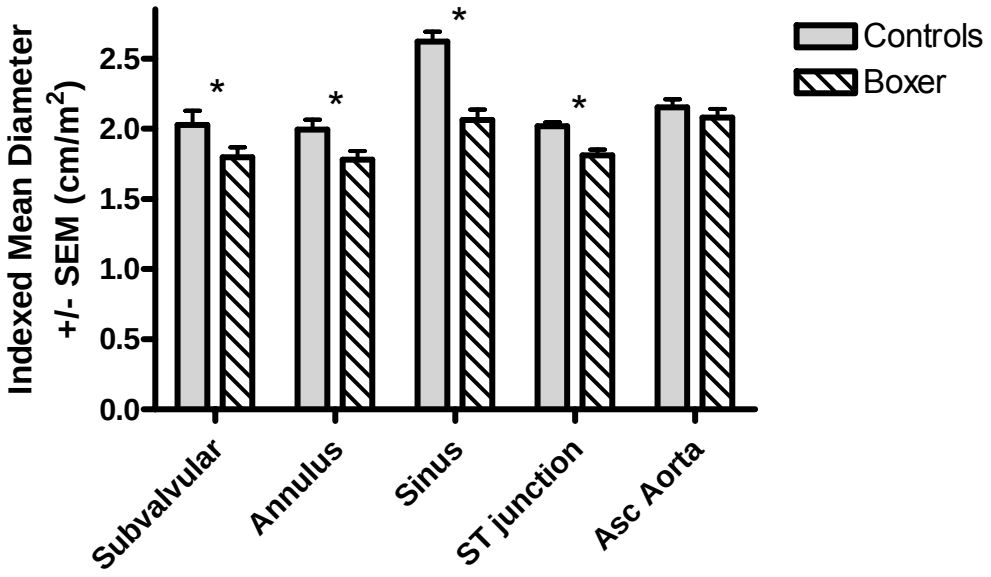

Figure 4.12: Indexed mean LVOT angiographic diameters +/- SEM.

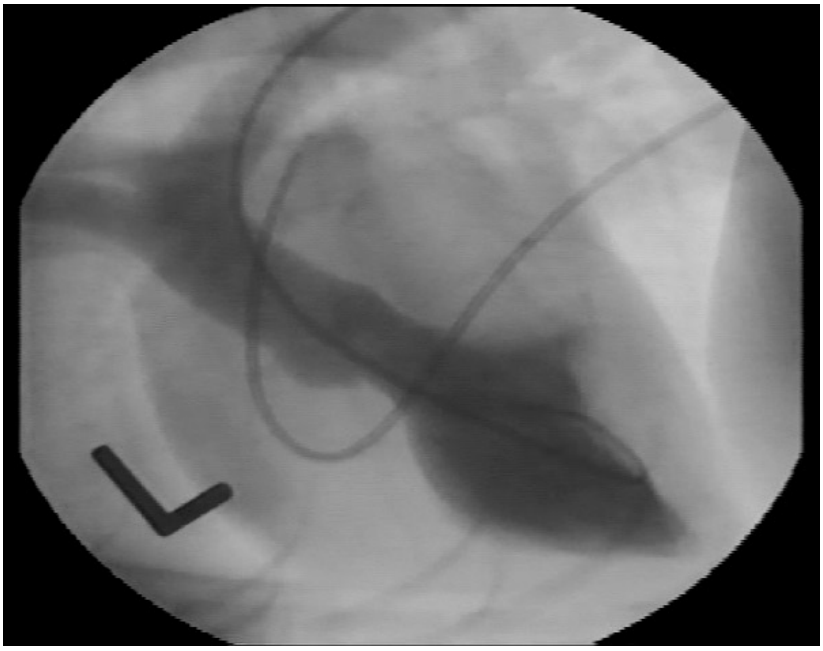

Figure 4.13: Control angiogram

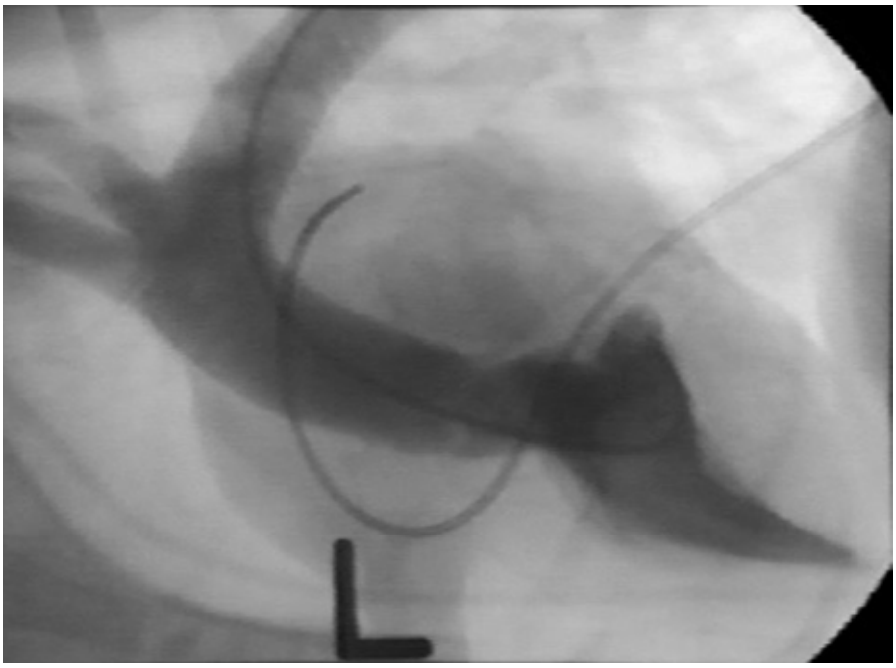

Figure 4.14: Boxer angiogram

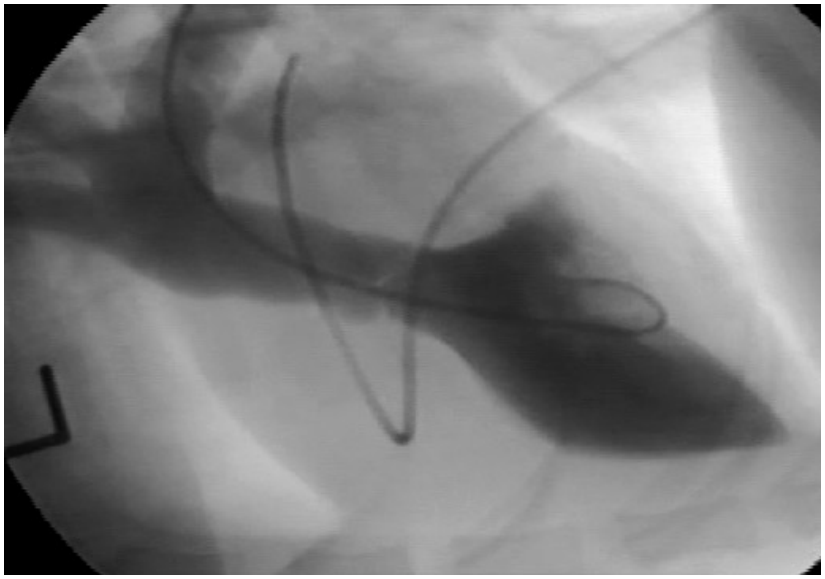

Figure 4.15: Boxer angiogram

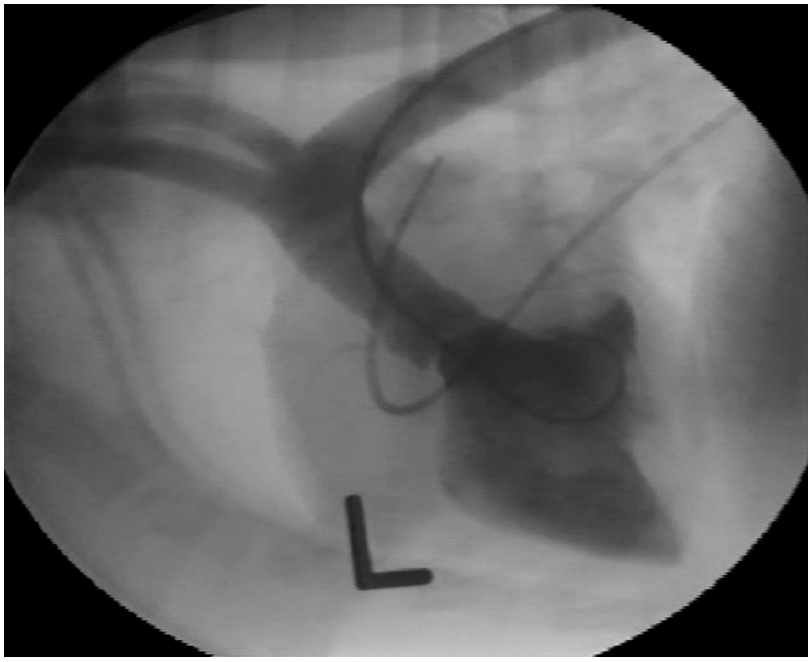

Figure 4.16: Boxer angiogram.

Discussion

The results of this study suggest boxers have a diffusely narrowed LVOT, based on angiography. These findings are consistent with those of chapter 3, which found smaller LVOT dimensions and higher outflow tract velocities in boxers with murmurs compared with non-boxer dogs. In this prior echocardiographic study, hemodynamic factors appeared to play a prominent role in determining ejection velocity and development of murmurs in boxers. A low correlation coefficient relating LVOT area to velocity suggested that less than 40% of the change in velocity or stroke distance might be explained by a change in LVOT area. In addition, there was a trend toward a higher SVI in boxers with murmurs compared to boxers without murmurs.

Hemodynamic manipulations in the current study were used to influence blood flow through the LVOT in a more controlled manner. Our goal was to simulate settings that might induce a physiologic murmur, as with an increase in stroke volume due to increased preload and/or sympathetic stimulation (increased contractility). Increased preload was simulated by injection of an iodinated contrast agent. No statistical differences in aortic velocity, echo PG, cath PG, CI, SVI, or HR were found following this manipulation, although numerically the mean values during the post-angiogram interval were higher than at anesthesia alone for all parameters. It is likely that 2 ml/kg of contrast did not correlate with a large enough osmotic effect to elevate preload dramatically. However, the latter was not measured. Alternatively, it is also

possible that preload does not have a large enough impact on ejection velocity and pressure gradients to be detected in this study design.

Increased sympathetic stimulation was simulated with two different doses of dobutamine infusion (2.5 and 5 μg/kg/min). During these time points, differences in echocardiographic parameters reached statistical significance between the groups. Aortic velocities and Doppler-derived pressure gradients were higher in boxers compared with control dogs at both dobutamine concentrations, while no differences between groups were detected during baseline ($p=0.11$) or anesthesia time points ($p=0.72$; Figure 4.1). Boxers also showed a trend toward an exaggerated response to dobutamine for the catheter-derived PG ($p=0.051$). These findings may indicate that boxers are more sensitive to sympathetic stimulation or that the accompanying increase in flow may be required in order to detect a difference between control dogs and the boxers with a smaller LVOT. The boxer trend toward a higher dobutamine-induced CI ($p=0.09$) and the more dramatic percent increase in SVI compared to controls (82% vs. 29%), further supports the possibility that boxers are more sensitive to dobutamine. Importantly, these findings help illustrate the effect of stroke volume on LVOT pressure gradients (Figure 4.10). However, mean Dob 5 stroke volume indices were very similar between the groups (79 vs. 80 ml/m^2). The different pressure gradients at this time point despite equivalent stroke volumes, supports the impact of a smaller area on the LVOT velocity and pressure gradients.

Despite substantial increases in flow across the LVOT with Dob 5 (82% increase in boxers), a peak to peak PG larger than 5 mmHg was present in only two dogs (one boxer at 5.6 mmHg and the other boxer at 30 mmHg). Surprisingly, the 30 mmHg peak to peak PG was generated by the boxer with the lowest baseline aortic velocity (1.96 m/s). Compared with the anesthesia time point, the SVI increased 164% at Dob 5 in this dog. It is important to note that pressure recordings were not obtained for the dog with the highest baseline aortic velocity at the Dob 5 interval. However, the Dob 2.5 peak to peak PG was only 4.7 mmHg, despite a relatively high SVI (85 ml/m^2). Peak to peak gradients would be expected in the setting of genuine stenosis. The failure to identify such gradients in this study may indicate true stenosis does not exist or that peak to peak PG are only identified in more severely stenotic lesions. Pressure recovery, associated with a gradual/streamlining stenosis (compared with a discrete stenosis), may also play a role. In this case, kinetic energy is not lost to turbulence and heat, but it is reconverted to pressure energy, such that no pressure drop is recorded, despite the presence of a stenosis and high velocity.[11]

In the interpretation of this data, we looked for a distinction between impulse gradients and stenotic gradients. Impulse gradients refer to early intraventricular or transvalvular gradients in the absence of obstruction that allow inertia to be overcome in order to achieve ejection of blood.[12] The transvalvular impulse gradients in normal individuals are small, early systolic, asymmetric, and may persist until mid-systole.[12,13,14] The early systolic

component is typically augmented by exercise, ß-adrenergic agonists, and long diastolic intervals. In exercising people, these gradients have been reported as high as 25 to 35 mmHg.[12,13] The average duration of the positive transvalvular impulse gradient in normal dogs was 45% of the ejection period, with a range of 27 to 63%.[14] In contrast, stenotic or obstructive gradients are typically symmetric and rounded in contour with a longer time to peak gradient and a positive gradient throughout ejection, compared with the usual reversal in gradients later in ejection without obstruction.[12,13] Convincing evidence of stenotic gradients were not identified in any of the dogs in this study. In addition, there were no statistical differences in the time to peak gradient, PG duration, ratio of time to peak PG to PG duration, and ratio of PG duration to ejection time. However, the Doppler-derived time to peak PG was significantly shorter in boxers compared with control dogs for the post-angio, Dob 2.5, and Dob 5 time points. An earlier peaking PG is more suggestive of an impulse gradient, whereas a later peaking gradient would be more compatible with aortic stenosis. The subjective appearance of the highest peak to peak PG was suggestive of a large impulse gradient (Figure 4.6).

As expected, the Doppler-derived PG statistically predicted the cath PG. However, the strength of the fit ($r^2 = 0.82$, $r = 0.9$) is not as good as that previously presented for dogs with aortic stenosis ($r = 0.99$).[9] This finding may reflect the differences in the population. The prior study was performed in dogs with moderate to severe aortic stenosis, wherein the assumptions of the simplified Bernoulli equation hold true. The current study examined dogs with

equivocal to mild aortic stenosis, where some assumptions of the Bernoulli equation may not be valid. Specifically, the simplified Bernoulli equation assumes that the velocity proximal to the obstruction (v1) is negligible, and therefore it is ignored. In most cases, v1 is usually less than 1 m/s (less than 4 mmHg), and this is usually trivial compared to the velocity across a moderate to severely stenotic region. However, in high flow states or with relatively low peak gradients, ignoring this variable can lead to an overestimation of the pressure drop.[15] In addition, the simplified Bernoulli equation assumes no frictional losses. This assumption is valid for discrete stenoses, although it may not be valid for long-segment stenoses.[15] The physiology of this type of stenosis may be relavent to the boxer, in light of the diffusely narrowed LVOT found on angiography.

For control dogs, the Bland Altman graphs revealed a trivial bias between echo and cath PG (-0.12 mmHg) with a modest 95% CI (-5.9 to 5.7 mmHg; Figure 4.11). This reflects overall excellent agreement between Doppler and catheter gradients in control dogs. For boxer dogs, the instantaneous echo PG was an average of 2.56 mmHg higher than the cath PG, and the 95% CI were also slightly wider than that for control dogs (-5.8 to 10.9 mmHg). An overestimation of approximately 11 mmHg in the Doppler PG compared with the catheter PG may be clinically important in a boxer with otherwise equivocal evidence of aortic stenosis. Despite the theoretical reasons listed above for a difference between these tests, the current study suggests slightly better overall agreement between Doppler and catheter PG, compared with the previously

reported bias. In that study, Doppler overestimated catheter PG with a mean bias of 9 mmHg and (as estimated from the graph) a 95% CI of –9 to 27 mmHg.[9]

Angiocardiographic dimensions in boxers were smaller over the entire LVOT compared with control dogs, with the exception of the ascending aorta location. These findings are consistent with our previous echocardiographic findings, although the angiographic data provide more convincing differences and greater detail in assessing the location of those differences. In chapter 3, pooled LVOT dimensions were larger in non-boxers compared to boxers with murmurs, and statistical differences between these groups reached statistical significance at the ST junction and the ascending aorta locations only.

The largest difference between the angiographic dimensions of boxers and controls was identified at the sinuses of Valsalva. In every boxer dog the sinuses were subjectively small. The ascending aorta was subjectively enlarged in half of the boxers and was approximately equal to the sinus dimension in the other half. In normal dogs, the sinuses of Valsalva are always wider than all other parts of the LVOT,[16,17] and early angiographic descriptions reported that an ascending aorta wider than the sinuses supports a diagnosis of aortic stenosis.[16] Yet, when the boxer ascending aorta is compared with the controls in this study, no difference is found, suggesting relative diminution of the sinuses rather than dilatation of the ascending aorta. In fact, echocardiographic data support a relatively smaller proximal ascending aorta

(chapter 3), while the objective angiographic data support a normal-sized distal ascending aorta.

As expected, the echocardiographic dimensions statistically predicted the angiographic dimensions. The strength of this relationship is good (r^2 = 0.75). A greater error might be expected with the angiographic dimensions due to the lower frame rate of image acquisition and the need for repeated calibration on every image. Bland Altman analysis indicates substantial overestimation of the echocardiographic dimensions compared with the angiogram, with a bias of 0.22 cm/m^2. Most likely, the angiographic dimensions were underestimated due a magnification artifact, as the reference marker for calibration was placed directly on the table instead of at the same elevation as the LVOT. Genuine differences in dimensions between a conscious unsedated dog (echocardiographic dimensions) and the anesthesized dog (angiogram dimensions) may have played a smaller role.

Limitations

The small number of dogs, along with exclusion of some of the dogs from the catheterization data, led to a lack of adequate power for many of the tests in this study.

The influence of anesthesia has well-recognized and depressive effects on ejection velocity, ventricular contractility, arterial blood pressure, cardiac index, and stroke volume index.[18] The anesthetic depth was subjectively assessed in order to maintain a light plane of anesthesia. Some differences between dogs would be anticipated, and there may be a difference between the

breeds in their response to sedatives and anesthesitics. No attempt was made to quantitate these potential differences. Additionally, it is possible that the depth of anesthesia changed during the course of the procedure, having varying effects at different measurement intervals. These variables may have prevented finding expected changes under different manipulations, but they should not have affected the comparison between echo and cath PG.

Whether the Hound dogs represented an adequate control group may be questioned. One of these dogs had mildly thickened aortic leaflets on 2-D echocardiography. Baseline examination for this dog, including a grade 2/6 left basilar ejection murmur and aortic velocity of 1.91 m/s, did not confirm the presence of aortic stenosis. Additionally, 4/6 controls had subcostal aortic velocities greater than 2 m/s. While trivial LVOT obstruction cannot be conclusively ruled out in these dogs, the rarity of AS in this breed makes such findings more compatible with physiologic changes.

In conclusion, this study found a diffusely narrowed LVOT in a small subset of boxer dogs with echocardiographic evidence of equivocal to mild aortic stenosis. The lack of supporting catheterization data, such as stenotic-type gradients or substantial peak to peak PG, may reflect a lack of obstruction despite morphologic differences in the LVOT, as may be suggested for the control dog with the imaging lesion. It remains uncertain if a diffusely narrowed LVOT represents a type of aortic stenosis in the boxer dog, with potential clinical and heritable consequences. Alternatively, it may be a breed characteristic independent of aortic stenosis and merely helps to explain the

high frequency of murmurs and elevated aortic velocity in boxer dogs. Nevertheless, the morphologic lesions detected on the echocardiogram and/or angiogram offer greater support to the possibility of genuine stenosis, and further comparisons between boxers without murmurs and those with equivocal evidence of aortic stenosis are indicated.

Footnotes

[a] GE Medical, Horten, Norway

[b] Millar Instruments, Houston, Texas

[c] Biopac MP 100 Data Acquisition System, Biopac Systems Inc, Santa Barbara, CA

[d] Series 7010 Monitor, Marquette Electronics Inc, Milwaukee, WI

[e] Omnipaque (iohexol), 240 mg I/ml; Amersham Health, Princeton, NJ

[f] Picker Clinix MP Plus, Picker International, Cleveland, OH

[g] Sigma Stat for Windows version 2.03, San Rafael, CA.

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CHAPTER 5

SITE OF MURMUR GENERATION ASSESSED BY INTRACARDIAC PHONOCARDIOGRAPHY

Introduction

Turbulence, describing a disturbance to laminar flow, is the primary factor in the generation of ejection murmurs.[1,2,3] The likelihood of turbulent flow is described by the Reynold's number with a larger number reflecting a greater tendency toward turbulence. This value is directly related to velocity, area, and fluid density and is inversely related to fluid viscosity.[1]

Healthy adult boxer dogs frequently demonstrate left basilar ejection murmurs on physical examination.[4,5,6] The data in chapters 2 and 3 confirm that murmur intensity correlates well with left ventricular outflow tract (LVOT) velocity, so it is likely that left basilar ejection murmurs originate somewhere in the LVOT. Although subvalvular aortic stenosis is commonly reported in boxer dogs,[7,8] valvular aortic stenosis has also been described.[9,10] Still other proposed explanations for these murmurs include hypoplasia of the LVOT (in the absence of a discrete stenosis)[11] or the presence of physiologic murmurs (in the absence of any morphologic lesions within the LVOT, as associated with increased stroke volume).[4,5,7-9,11,12]

The results of chapters 3 and 4 indicate a diffuse narrowing of the LVOT in boxer dogs, potentially independent of murmur status. Convincing evidence of a discrete stenosis have not been identified in most dogs with soft murmurs, and the murmurs in some dogs may simply reflect elevated stroke volume as suggested in chapter 3. This study was conducted to identify the site of the murmur generation and to provide additional information about the pathogeneisis of these murmurs.

Materials and Methods

Data for this study was collected concurrently with that of chapter 4. The subjects included the boxer cohort in chapter 4, representing 8 healthy adult dogs with left basilar ejection murmurs and aortic velocities between 2 and 3 m/s.

Baseline examination

Auscultation of each dog was conducted in a quiet room for a minimum of one minute. The intensity of the left basilar ejection murmur was recorded using the grading scheme outlined in chapter 1. Two-dimensional and Doppler echocardiography was performed as described in chapters 3 and 4.

Cardiac catheterization

Details regarding the anesthetic and catheterization procedure are presented in chapter 4.

Intracardiac phonocardiography

An 8 French straight-tipped Millar catheter with an end hole and micromanometer placed at the distal extent of the catheter was advanced retrograde into the left ventricle over a guidewire (0.032). Pressure waveforms from the distal micromanometer were filtered with a low pass of 60 Hertz and a high pass of 125 Hertz to create intracardiac phonocardiograms.

Phonocardiograms were recorded from 4 sites within the LVOT at each of the recording intervals: anesthesia, post-angiogram, Dob 2.5, and Dob 5. Locations were verified with a combination of fluoroscopy and the appearance of the pressure waveform (Figure 5.1). Location 1 corresponds to the proximal portion of the LVOT, judged by a left ventricular pressure waveform with the position of the catheter tip in the upper 1/2 of the left ventricle. Location 2 represents the subvalvular region and was identified by a ventricular waveform just below the location of exaggerated valve noise from aortic valve closure at S2 with a fluoroscopic position distal to location 1. Location 3 was at the aortic valve, judged by an aortic pressure waveform and prominent aortic valve noise on the phonocardiogram. Location 4 was at the proximal ascending aorta, with an aortic pressure waveform and a fluoroscopic position within the proximal 1/3 of the ascending aorta. During recording, the guidewire helped stabilize catheter position.

Murmur duration was measured from the onset of S1 to the end of the murmur and from the onset of the murmur to the end of the murmur (when the end of S1 and beginning of the murmur could be distinguished from one

another). In general, the onset of the murmur to the end of the murmur could only be measured for crescendo-decrescendo (diamond) shaped murmurs. The onset of the decrescendo shaped murmurs most often blended into S1, and the murmur duration could not be measured without including S1. Ratios of murmur duration to the duration of systole (from onset of S1 to the onset of S2) were also calculated. Maximal murmur amplitude was measured and the ratio of murmur amplitude to maximal S1 amplitude was calculated. A minimum of 5 closely-timed beats were measured and averaged for each parameter.

For each recording interval, the location (1 through 4) with the most prominent murmur, subjectively, was noted. Overall murmur amplitude and duration were the most important factors in assessing a murmur's prominence.

Statistics

Two way RM ANOVA was used to compare differences in murmur duration and amplitude across different locations, between different recording intervals, and to determine any interactions between locations and recording intervals.

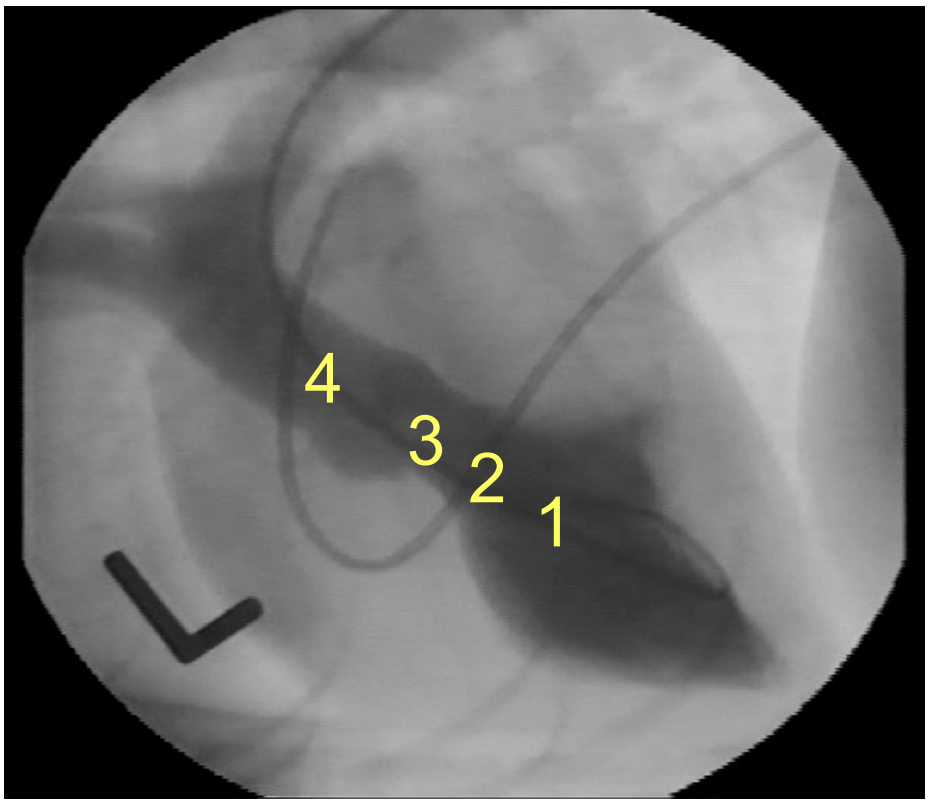

Figure 5.1: Anatomic locations for intracardiac phonocardiograms.

Results

Murmur assessment by Auscultation

Each of the boxer dogs had soft left basilar ejection murmurs during baseline evaluation. Two boxers had grade 1/6 murmurs, and their baseline aortic velocities were 1.96 and 2.1 m/s. Three boxers had grade 2/6 murmurs with baseline velocities of 2.04, 2.46, and 2.68 m/s. Three boxers had grade 3/6 murmurs, and their baseline aortic velocities were 2.46, 2.9, and 3.13 m/s.

The Dob 5 heart rate (119 bpm) was statistically higher than that at the pre-study (90 bpm), sedation (82 bpm), and anesthesia (94 bpm) timepoints ($p<0.025$), and the post-angiogram heart rate (103 bpm) was higher than the sedated heart rate ($p=0.03$).

Intracardiac phonocardiogram

A representative example of the phonocardiograms for each location is shown in Figure 5.2. Assessments of murmur duration, including S1 to end of murmur, murmur beginning to end, and their percentages of S1-S2, were all statistically longer at locations 3 and 4 compared with locations 1 and 2 ($p<0.013$, pooled data, Table 5.1). No statistical differences were found between locations 3 and 4 ($p>0.85$) or between locations 1 and 2 ($p>0.69$). Murmurs were numerically the longest at location 3, for both methods of measuring murmur duration. There was a trend for murmurs to be longer during Dob 5 ($p=0.06$), and this difference reached statistical significance when murmur duration was expressed as a percentage of S1-S2 ($p<0.04$).

Assessments of murmur intensity, including maximum murmur amplitude and the ratio of murmur amplitude to S1 amplitude, were greater at locations 3 and 4 compared with locations 1 and 2 ($p<0.016$, Table 5.1). Murmur amplitude and its ratio to S1 were not different between locations 3 and 4 ($p>0.6$) or between locations 1 and 2 ($p>0.9$). When individual time points were compared, no differences in murmur amplitude were found across locations for the anesthesia time point ($p>0.5$). During the post-angiogram time point, murmur amplitude at location 3 remained greater than location 1 ($p=0.04$). At Dob 2.5 and Dob 5 time points, location 4 murmurs were of higher amplitude than locations 1 and 2 ($p<0.008$). Location 3 murmurs were also higher than locations 1 and 2 at Dob 5 ($p<0.002$), while at Dob 2.5 location 3 murmurs were statistically higher than location 1 murmurs only ($p=0.046$, Table 5.1).

The murmur amplitude at locations 1 and 2 did not change with different time points ($p>0.55$). The amplitude of location 3 murmurs was higher at Dob 5 compared with anesthesia and post-angiogram time points. Location 4 murmurs were higher at Dob 5 compared with all other time points ($p<0.022$) and higher at Dob 2.5 compared with anesthesia ($p=0.02$). Location 3 murmurs represented numerically, not statistically, the highest murmur amplitude during the anesthesia and post-angiogram time points, while murmur amplitude was numerically the highest at location 4 during Dob 2.5 and Dob 5.

Subjective assessment of murmur generation

For all boxers and at all time points, intracardiac murmurs were judged to be most prominent at either location 3 or location 4. In no case was the murmur

most prominent at locations 1 or 2, and often no systolic murmur was evident at these locations. In three boxers, with baseline aortic velocities of 2.46, 2.9, and 3.13 m/s, the site of murmur generation was subjectively judged to be at location 4. In two dogs, with aortic velocities of 2.04 and 2.68 m/s, the site of murmur generation was location 3. For the other three boxers with velocities of 1.96, 2.1, and 2.46 m/s, murmurs subjectively appeared equally prominent at locations 3 and 4.

Each systolic murmur was described as either decrescendo (Decr) or crescendo-decrescendo (CD). For 2 boxers, the murmur shape at each location was unchanged by different time points. In the other 6 dogs, the murmur shape at individual locations was variable either within the same time point and/or between different time points. In the 3 dogs in which location 4 was identified as the subjective location of murmur generation, CD murmurs were consistently found at that location in 2 dogs while the other dog primarily demonstrated Decr murmurs at location 4. In the 2 dogs in which location 3 was found to be the subjective site of murmur generation, CD murmurs were consistently found in one dog, and CD was the primary murmur shape found in the other dog at location 3.

Diastolic murmurs were seen within the LVOT at positions 1 and/or 2 in 7/8 boxers. In 3 cases, they were described as holodiastolic, decrescendo murmurs, while in one case, only an early (proto) diastolic murmur was seen. Each of the dogs with color Doppler evidence of aortic regurgitation demonstrated diastolic murmurs.

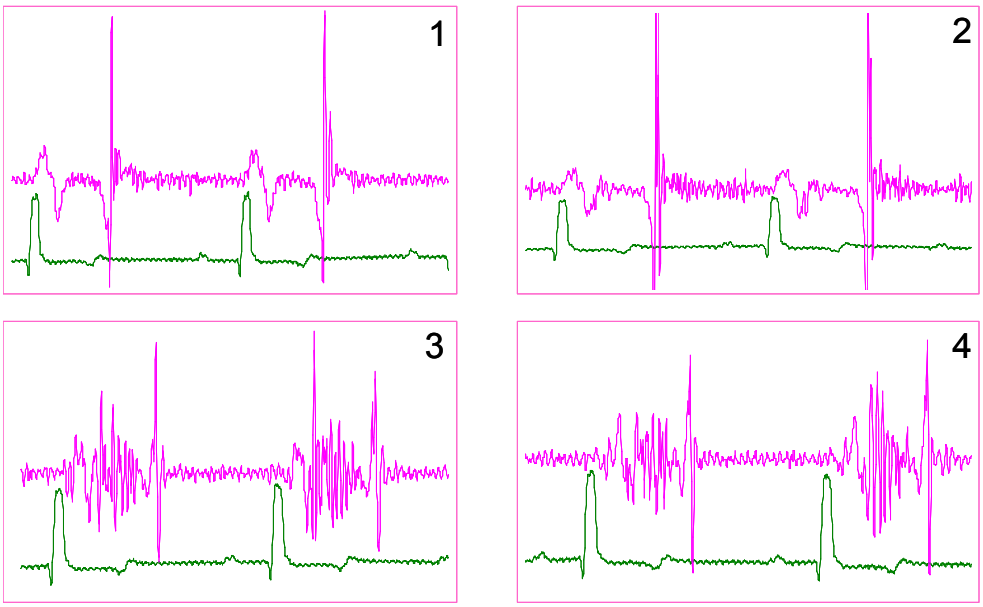

Figure 5.2: Representative intracardiac phonocardiograms.

	Left Ventricle Location 1		Subvalvular Location 2		At Aortic Valve Location 3		Ascending Aorta Location 4	
	Dur (ms)	Amp (Hz)	Dur (ms)	Amp (Hz)	Dur (ms)	Amp (Hz)	Dur (ms)	Amp (Hz)
Anesthesia	3 0 - 124	0 0 – 0.35	16 0 - 109	0 0 - 0	150 0 - 218	0.5 0 – 1.58	97 0 - 215	0.4 0 – 1.58
Post-angio	5 0 - 0	0 [a] 0 - 0	16 0 - 104	0.2 0 – 0.9	152 105-181	0.7 [b] 0.45-1.8	127 0 - 230	0.6 0.22-1.5
Dob 2.5	52 0 - 185	0.2 [a] 0 – 0.55	75 0 - 172	0.4 [a,b] 0 – 1.03	154 105-183	0.9 [b,c] 0.5 – 1.1	163 130-223	1.3 [c] 0.55-4.3
Dob 5	76 0 - 163	0.3 [a] 0 – 0.75	108 0 - 152	0.3 [a] 0 – 0.8	151 124-179	1.6 [b] 0.75-2.4	153 115-190	2.0 [b] 0.7-4.9

Table 5.1: Murmur duration and maximum murmur amplitiude (mean and range).

Discussion

Intracardiac phonocardiography indicates that the left basilar ejection murmurs present on physical examination in boxer dogs with mildly elevated aortic velocities originate within the distal LVOT, either at the aortic valve or in the proximal ascending aorta.

This finding is in agreement with the echocardiographic and angiographic data that showed a smaller LVOT in the region at and above the valve. In the echocardiographic study, the largest numerical differences in mean LVOT diameters between boxers with murmurs and control dogs were found at the sinotubular junction, proximal ascending aorta, and excursion of the aortic valve leaflets. In addition, boxers with murmurs had a more heterogeneous LVOT, wherein the subvalvular and aortic valve annulus dimensions were statistically larger than the leaflet excursion, sinotubular junction, and the ascending aorta (chapter 3). In the angiographic study, the largest numerical difference in LVOT diameters between boxers and controls was found at the sinus of Valsalva. Identifying the murmur at sites of relative narrowing offers further support that differences in LVOT area have a significant impact on murmur development in boxer dogs.

Turbulence plays a major role in the generation of ejection murmurs.[2,3] The local Reynold's number along with the size and shape of any obstacle to flow will influence the development of turbulence.[1] The Reynold's number is directly related to the area, velocity, and density, while it is inversely related to the viscosity.[1] As described by the Womersley (unsteadiness) parameter,

sufficient time is also required for turbulence to develop.[13] This concept is congruous with prior studies of intracardiac phonocardiography, which demonstrated the highest intensity murmurs are typically detected just distal to the location of the morphologic lesion creating the turbulence.[1,14,15]

Despite the time delay in turbulence development, prior studies have shown that maximum murmur intensity associated with subvalvular aortic stenosis is always found within below the aortic valve.[14] None of the boxers in this study demonstrated a murmur at the subvalvular locaton during the baseline or post-angiogram time points. During dobutamine infusion, subvalvular ejection murmurs were detected in 5/8 dogs, but these murmurs were always judged to be of lesser intensity and duration compared with the murmurs identified at more distal locations. These findings argue against the presence of occult subvalvular aortic stenosis as the cause of the murmurs in our boxers.

Prior studies in patients with valvular aortic stenosis found that intracardiac murmurs were consistently recorded from the ascending aorta, and maximum murmur intensity was found at an increasing distance distal to the aortic valve with increasing severity of stenosis.[2,16] In contrast, high stroke volume in the absence of aortic stenosis was associated with peak murmur intensity closer to the aortic valve and diminishing intensity within the ascending aorta.[2,16] Likewise, during isoproterenol infusion in normal dogs ejection murmurs were recorded just above the aortic valve.[17]

In this study, the two dogs with the highest velocities (2.9 and 3.13 m/s) also had aortic valve thickening on 2-D echo (chapter 3). For both dogs, the ascending aorta (location 4) was the subjective site of murmur generation. In the dog with the highest velocity, the murmur was also prominent at the aortic valve. During dobutamine infusion, CD murmurs were seen at locations 1 and 2, though they were more prominent above or at the valve. These findings may support the aortic valve as the cause of the murmur, and valve thickening is often a component of valvular stenosis. Alternatively, valve leaflets may be thickened without creating an obstruction, as flow disturbances associated with normal valves have also been described.[1,15,18]

The lowest baseline aortic velocities (1.96, 2.04, and 2.1 m/s) were associated with murmur prominence at the aortic valve (location 3) for one dog. For the other two dogs, intracardiac murmurs appeared equally prominent at both the aortic valve and ascending aorta locations. Interestingly, the highest peak to peak gradient (chapter 4) was associated with a murmur most prominent at the aortic valve. This location supports the role of an increased stroke volume in the generation of some murmurs.

In addition to turbulence, vortices have also been demonstrated in the region of a normal aortic valve under normal flow conditions. In normal situations, this flow disturbance lies predominantly behind the aortic leaflets within the sinuses of Valsalva leaving the main flow unobstructed.[1,15] This 'expected' flow disturbance might not be as isolated from the main aortic flow in the setting of a smaller sinus of Valsalva. In this manner, the smaller sinuses

identified in each of our boxer dogs may predispose to development of an ejection murmur, potentially independent of all other factors related to LVOT area or stroke volume.

The high number of diastolic murmurs (7/8 dogs) most likely reflects a high prevalence of aortic regurgitation, particularly when such murmurs were identified at position 2. Four of the dogs had a trivial to small amount aortic regurgitation identified on baseline Doppler echocardiography (chapter 4), and it is possible that this technique underestimated the severity and number of dogs affected. However, catheter-induced aortic regurgitation is also a likely explanation. Protodiastolic murmurs at position 1 may also reflect vigorous left ventricular filling, as this type of diastolic murmur often appeared more prominent during dobutamine infusion.

The results of this study identified the distal LVOT as the site of murmur generation for each of the boxer dogs with soft left basilar ejection murmurs on physical examination. These findings support both area differences at the valvular and supravalvular region and increased stroke volume as major determinants of ejection murmurs in boxer dogs.

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CHAPTER 6

SUMMARY AND CONCLUSION

The goal of this project was to investigate the etiology of a commonly encountered scenerio in clinical veterinary cardiology. Soft left basilar ejection murmurs are frequently auscultated in otherwise healthy, adult boxer dogs. This finding prompts echocardiographic evaluation to identify the cause and importance of the murmur. Even after the echocardiogram, however, the diagnosis remains uncertain for a substantial proportion of these boxer dogs. Many dogs have a trivial to mild elevation in left ventricular outflow tract (LVOT) velocities. Such findings may be physiologic in origin or they may be compatible with aortic stenosis. For breeders this distinction is critical, as aortic stenosis has been proven to be a heritable defect in the Newfoundland breed and is believed to be inherited in the boxer dog as well.

The relationship between the murmur grade and the LVOT velocity was investigated in 201 boxer dogs. The results confirmed clinical suspicions that murmur presence and grade are helpful in predicting the LVOT velocity by Doppler echocardiography. The presence of a left basilar ejection murmur was 87% sensitive and 66% specific in the identification of a LVOT velocity > 2 m/s. These results also confirmed the scope of the problem in that over 50% of

healthy adult boxers have typical murmurs, 42% have LVOT velocities compatible with possible aortic stenosis, and 36% have both typical murmurs and elevated aortic velocities. On a one year follow up examination, changes in murmur intensity and LVOT velocity were not clinically important for most dogs. These results validate the clinical approach of using ausculation to screen dogs prior to echocardiography. The remainder of the project aimed to evaluate the LVOT in the dogs that remained in the equivocal status following these standard testing used in the clinical examination.

Detailed echocardiography did not identify any subjective differences between boxers with murmurs and boxers without murmurs. However, indexed LVOT areas measured smaller for boxer dogs, independent of murmur status. Additionally, there was a trend for boxers with murmurs to have a higher stroke volume compared to boxers without murmurs. These findings indicated that boxers are predisposed to ejection murmurs due to a smaller LVOT, but murmur generation and elevated LVOT velocities were also strongly influenced by stroke volume.

Angiography demonstrated diffusely smaller indexed LVOT dimensions in boxer dogs, with a substantial relative reduction in the width of the sinus of Valsalva. However, the absence of both stenotic-type pressure gradients and substantial peak to peak gradients argues against the presence of an obstructed LVOT and supports the role stroke volume plays in determining pressure gradients across the LVOT.

Intracardiac phonocardiography identified the distal LVOT, either the aortic valve annulus or the proximal ascending aorta, as the site of murmur generation for all boxer dogs with soft left basilar ejection murmurs. This finding argues against the presence of occult subvalvular aortic stenosis and is compatible with both an elevated stroke volume and the smaller LVOT identified on the echocardiograms and angiograms.

The common problem of left basilar ejection murmurs in healthy adult boxer dogs appears to be multi-factorial. A diffusely smaller LVOT was found in all boxers investigated, while a larger stroke volume was found in those with a cardiac murmur. It remains uncertain if this structural abnormality is a component of aortic stenosis in the boxer dog with heritable and clinical consequences, or if it merely is a predisposing factor for the development of a murmur and elevated LVOT velocity, independent of risk. Nevertheless, these results should provide a foundation for more studies on the etiology, pathology and long-term clinical status of this clinical problem.

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