Comparison of peak flow velocity through the left ventricular outflow tract and effective orifice area indexed to body surface area in Golden Retriever puppies to predict development of subaortic stenosis in adult dogs

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Objective—To evaluate the usefulness of Doppler-derived peak flow velocity through the left ventricular outflow tract (LVOT Vmax) and effective orifice area indexed to body surface area (EOAi) in puppies to predict development of subaortic stenosis (SAS) in the same dogs as adults.

Design—Prospective, longitudinal, observational study.

Animals—38 Golden Retrievers.

Procedures—Cardiac auscultation and echocardiography were performed on 2- to 6-month-old puppies, then repeated at 12 to 18 months. Subaortic stenosis was diagnosed when LVOT Vmax was ≥ 2.3 m/s in adult dogs with left basilar systolic murmurs.

Results—All puppies with EOAi < 1.46 cm^2/m^2 had SAS as adults. All adults with EOAi < 1.29 cm^2/m^2 had SAS. An LVOT Vmax > 2.3 m/s in puppyhood was 63% sensitive and 100% specific for SAS in adulthood. In puppies, LVOT Vmax was more strongly associated with a future diagnosis of SAS (area under the curve [AUC], 0.89) than was EOAi (AUC, 0.80). In puppies, the combination of LVOT Vmax and EOAi yielded slightly higher sensitivity (69%) and specificity (100%) for adult SAS than did LVOT Vmax alone. In unaffected and affected dogs, LVOT Vmax increased significantly from puppyhood to adulthood but EOAi did not.

Conclusions and Clinical Relevance—In Golden Retriever puppies, LVOT Vmax > 2.3 m/s and EOAi < 1.46 cm^2/m^2 were both associated with a diagnosis of SAS at adulthood. The combination of these 2 criteria may result in higher sensitivity for SAS screening. Unlike LVOT Vmax, EOAi did not change during growth in either unaffected Golden Retrievers or those with SAS. (*J Am Vet Med Assoc* 2014;245:1367–1374)

Subaortic stenosis is one of the most common congenital heart defects in Golden Retrievers.[1,2] It appears to be inherited in this breed.[3] Subaortic stenosis is a progressive defect, and the stenosis phenotype typically develops in the first months after birth.[1,4–9] Because of its progressive nature, early detection of SAS remains challenging, and assessment prior to 1 year of age may underestimate its severity or fail to identify its existence altogether.[6] Whereas mild and moderate forms of the disease are associated with a good prognosis, many dogs with severe SAS die suddenly, often within the first 3 years after birth.[1]

Definitive diagnosis of SAS typically involves Doppler echocardiography, but clear criteria for SAS screening in dogs are controversial.[1,3,6] Despite being highly flow dependent, measurement of LVOT Vmax or left ventricular-to-aortic pressure gradient is commonly used as the principal diagnostic means to assess the severity of SAS and establish a prognosis.[4,10] In human medicine, valvular EOA is often considered a better indicator of aortic stenosis severity than LVOT Vmax or

Abbreviations

AI	Aortic insufficiency
AUC	Area under the curve
EOA	Effective orifice area
EOAi	Effective orifice area indexed to body surface area
LVOT	Left ventricular outflow tract
LVOT Vmax	Doppler-derived peak flow velocity through the left ventricular outflow tract
NPV	Negative predictive value
PPV	Positive predictive value
ROC	Receiver operating characteristic
RVOT	Right ventricular outflow tract
SAS	Subaortic stenosis
Vmax	Doppler-derived peak flow velocity
VTI	Velocity-time integral

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pressure gradient because it is less flow dependent.[11–13] It has been demonstrated that the LVOT EOAi is an accurate indicator of SAS severity in dogs.[14] However, because of the potentially progressive nature of the disease during growth, the usefulness of EOAi in puppies as an early marker for SAS is unknown. The aim of the study reported here was to evaluate and compare the LVOT Vmax and EOAi measured in Golden Retriever puppies as a predictor of SAS at adulthood.

Materials and Methods

Animals—Golden Retriever puppies aged 2 to 6 months were recruited from breeders and clients of a veterinary teaching hospital between January 2009 and January 2013. The ethics committee, in accordance with Canadian Council of Animal Welfare guidelines, approved the study protocol. All owners signed a consent form.

Each study animal was evaluated with confirmation of identification (tattoo or microchip), physical examination, measurement of Doppler-derived systolic blood pressure,[a] and echocardiography (baseline examination). Blood pressure measurements were obtained as previously described.[15] Animals were placed in right-sided lateral recumbency, and blood pressure measurements were taken on the left forelimb. The first measurement was discarded, and the mean of the next 5 consecutive measurements was calculated. All parts of the baseline examination were repeated when each dog reached 12 to 18 months of age (follow-up examination). Dogs were excluded from the study if, on either visit, they had additional cardiac malformations or anomalies (including severe valvular regurgitation), systemic hypertension (defined as mean systolic arterial blood pressure > 160 mm Hg), overt signs of illness, lack of identifiable tattoo or microchip or inconsistency in identification from the first to the second visits, noncooperation during evaluation, or failure to return for the follow-up examination.

Echocardiography—The echocardiographic examination (2-D, M-mode, and Doppler blood flow) was performed by 1 of 2 experienced examiners (MCB or EC) using an ultrasound unit[b] equipped with either a 1.5- to 4.0-MHz or 3.5- to 8.0-MHz phased-array transducer and simultaneous ECG display. Echocardiographic measurements were acquired in a preset order.[16] To encourage cooperation through postprandial relaxation, owners or breeders were asked to feed puppies immediately prior to baseline echocardiography examination; no sedatives were administered. During echocardiography, all dogs were positioned in lateral recumbency while obtaining standard views (right parasternal, left apical, and subcostal).[16] Echocardiograms were then reviewed at a later date in 1 session, a minimum of 2 months after examination and acquisition of echocardiographic images, by a single board-certified cardiologist (EC) who was not aware of the identity or physical examination findings of individual dogs at the time of review. Calculation of EOA was performed offline at a later time by use of the continuity equation. The continuity equation specifies that flow through a given area of a conduit must equal flow through a contiguous area of the same conduit over a fixed period. Accordingly, the stroke volume ejected through the LVOT is equal to the flow that passes through the EOA of the aortic valve as follows:

$$SV = A_{LVOT} \times VTI_{LVOT} = EOA \times VTI_{Ao}$$

where SV is stroke volume, A_{LVOT} is the cross-sectional area of the LVOT, VTI_{LVOT} is the VTI of the LVOT pulsed Doppler signal, and VTI_{Ao} is the VTI of the aortic jet continuous-wave Doppler signal.

Considering that obstruction to the left ventricular outflow in dogs most commonly is subvalvular, the stroke volume was measured from the RVOT,[11,12,14] as proposed and validated previously.[17,18] In the specific context of SAS, VTI_{Ao} is the VTI of the subaortic (rather than aortic) jet continuous-wave Doppler signal.

The EOA was calculated by use of the following equation:

$$EOA = \frac{SV}{VTI_{LVOT}} = \frac{A_{RVOT} \times VTI_{RVOT}}{VTI_{LVOT}}$$

where A_{RVOT} is the cross-sectional area of the RVOT.

The A_{RVOT} was calculated by use of the following equation:

$$A_{RVOT} = D_{RVOT}^2 \times \frac{\pi}{4}$$

where D_{RVOT} is the RVOT diameter measured from the right parasternal short-axis view at the base of the pulmonic valve leaflets, from inner edge to inner edge during early systole.

The Vmax through the RVOT was obtained from the same view by means of pulsed-wave Doppler, with the sample volume positioned just beneath the pulmonic valve. The spectral envelopes were traced for measurement of VTI_{RVOT} (ie, the VTI of the RVOT). A minimum of 3 consecutive cardiac cycles were measured and the mean calculated to minimize the variation of VTI caused by the respiratory cycle. The continuous-wave Doppler velocity signal of LVOT flow was recorded from the subcostal view at an intercept angle < 20°.[19] Three cardiac cycles with the highest velocity and best-defined outer velocity envelope were selected for measurement and calculation of mean Vmax and VTI. Heart rate was recorded from the simultaneously captured ECG on the Doppler image used for calculating VTI. Values obtained were indexed to body surface area to correct for somatotype variation, with body surface area calculated from the following equation on the basis of body weight[20,21] as follows:

$$\text{Body surface area} = \left(10.1 \times BW^{2/3}\right) \times 10^{-4}$$

where BW is body weight in grams.

Aortic and pulmonic insufficiency were assessed by assigning a subjective grade to the width of the regurgitant jet at its origin, relative to the dimension of the RVOT or LVOT, as has been previously described.[4,22] Mitral and tricuspid regurgitation, when present, were assessed by a semiquantitative method, comparing the maximal regurgitant jet area with the left or right atrial

size. Jet areas < 20%, 20% to 40%, and > 40% of the atrial area corresponded to mild, moderate, and severe regurgitation, respectively.[22]

The presence or absence of SAS was determined from results obtained at follow-up. Adult dogs with both a left basilar systolic ejection murmur and LVOT Vmax ≥ 2.3 m/s were considered to have SAS,[6] whereas adult dogs with an LVOT Vmax < 2.3 m/s were classified as healthy (unaffected dogs). To attempt to separate dogs with SAS from clinically normal dogs with greater accuracy, a secondary analysis was performed. Adult dogs with an LVOT Vmax < 2.0 m/s were considered to be clinically normal and those with an LVOT Vmax > 2.5 m/s were considered to have SAS. Adult dogs with an LVOT Vmax of 2.0 to 2.5 m/s were empirically included in an equivocal group.[23] Dogs in the equivocal group were excluded.

Statistical analysis—The relationship between sex and disease status was examined by means of an exact χ^2 test. The relationship between age and weight and disease status was examined with a Student *t* test. The relationship between mean EOAi at the 2 time points was investigated with a repeated-measures linear model, with age class as a within-subject factor and disease status as a between-subject factor. A similar model was used for mean LVOT Vmax and mean heart rates. Following this analysis, contrasts between pairs of means were made, adjusting the comparison-wise α level with the sequential Bonferroni procedure for multiple comparisons. Two secondary similar analyses were performed for EOAi and LVOT Vmax after excluding animals in the equivocal group (adult dogs with an LVOT Vmax of 2.0 to 2.5 m/s) and for EOAi after excluding the 6 values with AI classified as moderate because it could potentially underestimate the EOAi. To examine the relationship between the mean LVOT Vmax and mean EOAi in adults, a segmental regression analysis was used for puppies and adults separately. This regression fit 2 adjoining regression lines with different slopes and intercepts and identified the EOAi at the point at which the 2 curves diverged. On the basis of ROC curve analysis, sensitivity and specificity were established with respect to disease status, and different thresholds of EOAi or LVOT Vmax were established for puppies and adults separately. The PPV and NPV were calculated from the thresholds established by ROC curve analysis. Statistical analysis was performed with the aid of commercial software.[c] The level of significance was set at $\alpha = 0.05$.

Results

Study dogs—Fifty-two Golden Retriever puppies were recruited and evaluated at baseline. Two were excluded because of concomitant congenital cardiac abnormalities (one had a ventricular septal defect, and the other had tricuspid dysplasia). Eleven puppies were lost to follow-up and 1 died of a noncardiac cause (ie, was hit by a car), leaving 38 dogs (25 females and 13 males) that were reevaluated as adults and retained in this study. At baseline, the median age of puppies was 16 weeks (range, 8 to 23 weeks) and body weight was 10.5 kg (23.1 lb), with a range of 3.8 to 25.1 kg (8.4 to 55.2 lb). At follow-up, the median age of dogs was 14 months (range, 12 to 16 months) and body weight was 28.1 kg (61.8 lb), with a range of 16 to 36.4 kg (35.2 to 80.1 lb). Twenty-two of 38 (58%) were assessed as unaffected at adulthood (16 females and 6 males), whereas 16 (42%) dogs had SAS (9 females and 7 males; from several litters and 7 breeders). No dog had overt clinical signs of decompensated SAS such as exercise intolerance, syncope, or dyspnea at baseline, nor were any deaths directly attributable to SAS during the study period. Clinically normal puppies were significantly ($P = 0.03$) older (mean age, 18 weeks) than those with SAS (mean age, 12 weeks). There was no significant ($P = 0.08$) difference in body weight between the clinically normal puppies and those with SAS. There was no association between sex and disease status ($P = 0.32$). The equivocal group (adult dogs with LVOT Vmax of 2.0 to 2.5 m/s) included 17 dogs, which were excluded from the secondary analysis. Among these 17 dogs, 3 had an LVOT Vmax of 2.3 to 2.5 m/s and 14 had an LVOT Vmax of 2.0 to 2.29 m/s at adulthood.

Cardiac auscultation—Twenty-one of the 38 (55%) puppies had a heart murmur at baseline, compared with 27 (71%) on follow-up. Of dogs with SAS, 12 of 16 puppies had a heart murmur at baseline (I/VI, n = 1; II/VI, 3; III/VI, 8); the other 4 dogs were free of heart murmurs at baseline and developed a grade I or II heart murmur at follow-up. Nine of 22 (41%) puppies in the unaffected group had a heart murmur (I/VI, n = 7; II/VI, 2) at baseline, 3 of which resolved by young adulthood, suggesting juvenile heart murmurs. The remaining 6 all had grade I of VI heart murmurs both on baseline examination and at follow-up; the murmurs were considered to be physiologic, given that no structural abnormalities were found on Doppler echocardiography.

Echocardiographic measurements—Of the 16 dogs with SAS, 10 had an LVOT Vmax ≥ 2.3 m/s at baseline, and by definition, the remaining 6 only had evidence of SAS at follow-up. In all dogs with LVOT Vmax ≥ 2.3 m/s at baseline, LVOT Vmax remained ≥ 2.3 m/s at follow-up. Of dogs with SAS, mean ± SD LVOT Vmax was 2.63 ± 0.9 m/s at baseline, compared with 3.33 ± 0.9 m/s at follow-up (**Table 1**).

Dogs with SAS had significantly lower EOAi and higher LVOT Vmax, compared with unaffected dogs, both

Table 1—Mean ± SD Doppler echocardiographic data for unaffected Golden Retrievers and Golden Retrievers with SAS at 2 periods of examination: baseline (age 2 to 6 months) and follow-up (age 12 to 18 months).

	Baseline		Follow-up	
Variable	**Unaffected (n = 22)**	**SAS (n = 16)**	**Unaffected (n = 22)**	**SAS (n = 16)**
EOAi (cm^2/m^2)	2.16 ± 0.58*	1.60 ± 0.5*	2.14 ± 0.54*	1.41 ± 0.48*
LVOT Vmax (m/s)	1.70 ± 0.26*†	2.63 ± 0.91*†	1.99 ± 0.24*†	3.33 ± 0.92*†
Maximum pressure gradient (mm Hg)	11.79 ± 3.72*†	30.79 ± 22.9*†	16.02 ± 3.56*†	47.83 ± 26.94*†

*Value is significantly ($P < 0.05$) different between unaffected dogs and dogs with SAS at the same period of examination. †Value is significantly ($P < 0.05$) different between the 2 periods of examination for the same group.

as puppies ($P = 0.002$ and $P = 0.005$, respectively) and as adults ($P < 0.001$ for both; **Figures 1 and 2**). A significant ($P < 0.001$) correlation ($R^2 = 0.78$) was observed between LVOT Vmax at baseline and LVOT Vmax at follow-up. A similar correlation ($R^2 = 0.71$) was found for EOAi between baseline and follow-up ($P < 0.001$). Mean LVOT Vmax was significantly higher at follow-up than at baseline in both unaffected dogs ($P < 0.001$) and dogs with SAS ($P = 0.04$). Conversely, the EOAi did not change significantly between baseline and follow-up in either unaffected dogs ($P = 0.88$) or dogs with SAS ($P = 0.21$). In the secondary analysis, excluding dogs in the equivocal group, EOAi was still significantly lower in dogs with SAS than in unaffected dogs, both at baseline ($P < 0.001$) and on follow-up ($P = 0.003$). The EOAi also did not change significantly ($P = 0.3$) from baseline to follow-up evaluation in either unaffected dogs or dogs with SAS. All adult unaffected adult dogs had an EOAi > 1.29 cm^2/m^2; all of these dogs had had an EOAi > 1.46 cm^2/m^2 at baseline.

Aortic insufficiency was found in 14 of 16 dogs with SAS at follow-up (mild, 9; moderate, 5), compared with 8 of 22 (36%; all mild) unaffected dogs ($P < 0.001$). At baseline, AI also was more prevalent ($P = 0.004$) in dogs with SAS (9/16 [56%]; mild, 7; moderate, 2) than in the unaffected dogs (5/22 [23%]; all mild). Aortic insufficiency severity progressed significantly between baseline and follow-up in dogs with SAS ($P = 0.01$) but not in unaffected dogs ($P = 0.32$).

At follow-up, mean heart rate was not significantly ($P = 0.34$) different between dogs with SAS (92 ± 16 beats/min) and unaffected dogs (100 ± 15 beats/min). Similar ($P = 0.99$) results were obtained at baseline for dogs with SAS (126 ± 22 beats/min) and unaffected dogs (127 ± 18 beats/min). The relationship between LVOT Vmax and heart rate was not significant, either at baseline ($P = 0.69$) or at follow-up ($P = 0.1$).

Relationship between EOAi and Vmax—There was a strong, nonlinear relationship ($P < 0.001$; $r^2 = 66\%$) between EOAi and LVOT Vmax in adult dogs at follow-up (**Figure 3**). The same relationship ($P < 0.001$; $r^2 = 71\%$) was observed between EOAi measured at baseline and LVOT Vmax recorded at follow-up (**Figure 4**).

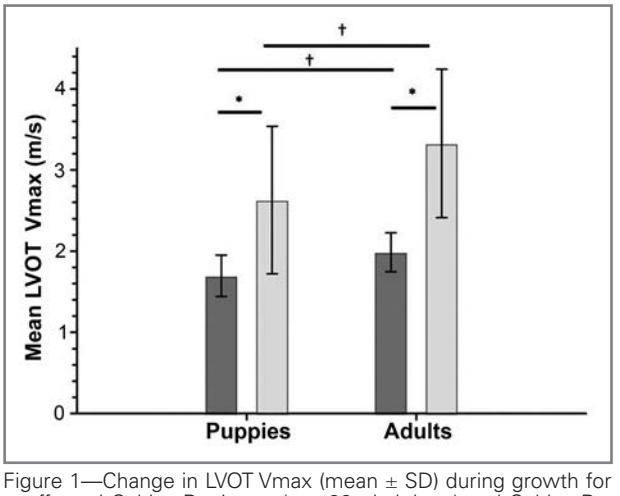

Figure 1—Change in LVOT Vmax (mean ± SD) during growth for unaffected Golden Retrievers (n = 22; dark bars) and Golden Retrievers with SAS (16; light bars). *Significantly ($P < 0.05$) different values between unaffected dogs and dogs with SAS at the same period of examination. †Significantly ($P < 0.05$) different values between the 2 periods of examination for the same dog group.

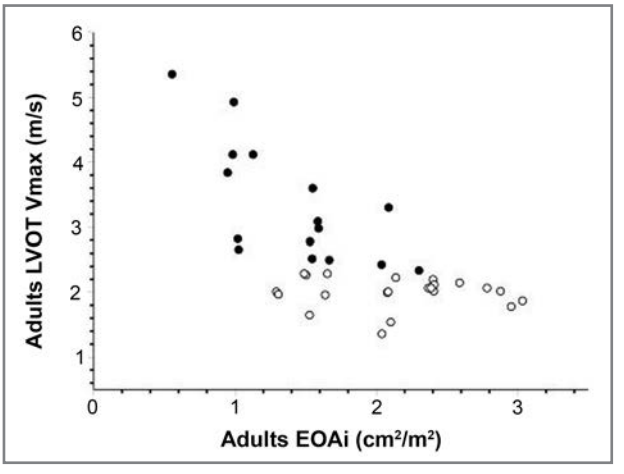

Figure 3—Relationship between EOAi and LVOT Vmax in adult dogs with SAS (n = 16; closed circles) and unaffected dogs (22; open circles). There was a strong, nonlinear relationship between EOAi and LVOT Vmax for all adult dogs ($P < 0.001$; $r^2 = 66\%$).

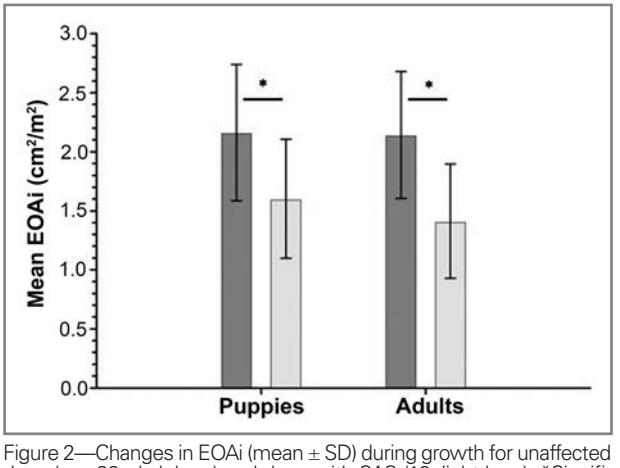

Figure 2—Changes in EOAi (mean ± SD) during growth for unaffected dogs (n = 22; dark bars) and dogs with SAS (16; light bars). *Significantly ($P < 0.05$) different values between unaffected dogs and dogs with SAS at the same period of examination.

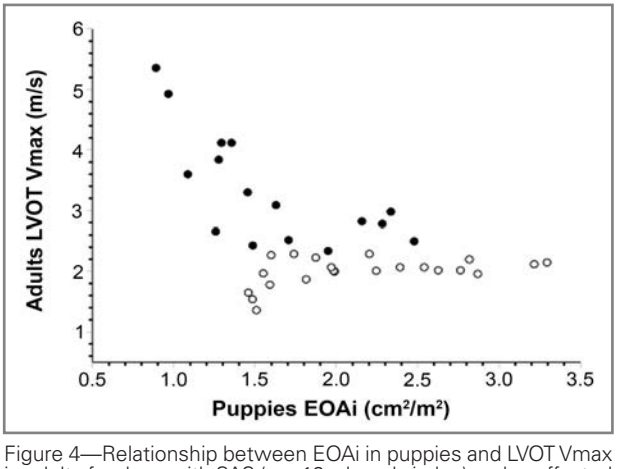

Figure 4—Relationship between EOAi in puppies and LVOT Vmax in adults for dogs with SAS (n = 16; closed circles) and unaffected dogs (22; open circles). There was a strong, nonlinear relationship between EOAi and LVOT Vmax for all dogs ($P < 0.001$; $r^2 = 71\%$).

Usefulness of EOAi versus LVOT Vmax for early detection of SAS—The ROC curve analysis revealed that the global predictive value of EOAi at baseline was good for having a diagnosis of SAS at follow-up (AUC, 0.80; **Figure 5**). The best cutoff was obtained at an EOAi of 1.49 cm^2/m^2 (sensitivity, 56%; specificity, 90%; PPV, 81%; NPV, 74%; Table 1). All study dogs with an EOAi < 1.46 cm^2/m^2 at baseline met the criteria for SAS on follow-up (sensitivity, 50%; specificity, 100%; PPV, 100%; NPV, 73%). Conversely, the maximal NPV for SAS was obtained with an EOAi of 2.49 cm^2/m^2 (sensitivity, 100%; specificity, 29%; PPV, 52%; NPV, 100%).

These results were compared with the predictive value of LVOT Vmax at baseline for meeting the criteria for SAS on follow-up. The ROC curve analysis revealed better predictability for LVOT Vmax (AUC, 0.89; Figure 5), compared with EOAi, and the best LVOT max cutoff at baseline was 2.25 m/s (sensitivity, 63%; specificity, 91%; PPV, 83%; NPV, 77%). The maximal PPV for meeting the criteria for SAS at follow-up was reached when LVOT Vmax was > 2.3 m/s at baseline (sensitivity, 63%; specificity, 100%; PPV, 100%; NPV, 79%). Conversely, the maximal NPV was found at an LVOT Vmax of 1.57 m/s (sensitivity, 100%; specificity, 32%; PPV, 52%; NPV, 100%) because all dogs with LVOT Vmax equal or below that cutoff were in the unaffected group at follow-up.

Although global accuracy to predict SAS was not better for EOAi than LVOT Vmax alone, a combination of LVOT Vmax and EOAi results yielded slightly higher sensitivity (69%) for detecting SAS at baseline evaluation than use of EOAi or LVOT Vmax alone (63% and 50%, respectively; **Table 2**).

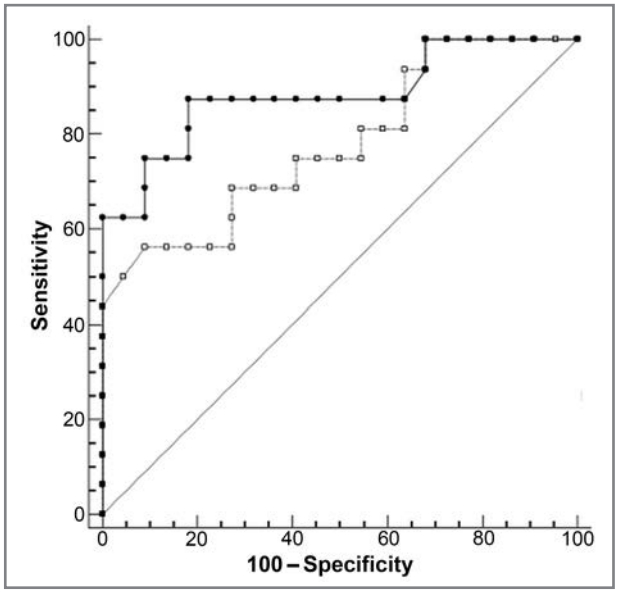

Figure 5—Comparison of ROC curve analysis of the predictive value of baseline LVOT Vmax (closed squares; AUC = 0.89; $P < 0.001$) and EOAi (open squares; AUC = 0.80; $P < 0.001$) measurements for meeting the criteria for SAS at follow-up in 38 dogs.

Discussion

Assessment of SAS is challenging in young animals because the condition may be clinically silent in puppies and only become apparent as the animal matures.[4] Newer diagnostic approaches that identify puppies at risk of developing SAS could be useful to improve recommendations veterinarians make to breeders trying to eliminate this defect from their breeding stock.

Previously, EOAi has been found to be significantly lower in dogs with SAS than in unaffected dogs, and a low EOAi has been strongly associated with the occurrence of adverse events including syncope, episodic weakness, and ventricular arrhythmias.[14] In the present study, 100% sensitivity and specificity cutoffs for EOAi were 2.48 and 1.46 cm^2/m^2, respectively. Indeed, all puppies with an EOAi < 1.46 cm^2/m^2 met the criteria for SAS as adults. However, for dogs with an EOAi between 1.46 and 2.48 cm^2/m^2 as puppies, the use of EOAi alone was not reliable for predicting the presence or absence of SAS at adulthood. Seven of 16 dogs could have been classified as affected (EOAi < 1.46 cm^2/m^2) but only 7 of 22 (32%) as unaffected (EOAi > 2.48 cm^2/m^2) on the basis of EOAi alone. The remaining 9 puppies with SAS and 18 unaffected puppies would have been classified as equivocal on the basis of EOAi alone.

All adult dogs without SAS had an EOAi ≥ 1.29 cm^2/m^2, which is similar to the previously reported value for unaffected dogs of various breeds.[14] However, the specificity of this finding is suboptimal because some adult dogs with SAS also had an EOAi > 1.29 cm^2/m^2. For adult dogs, 100% sensitivity and specificity cutoffs were 2.3 and 1.29 cm^2/m^2, respectively. All adult dogs with an EOAi < 1.29 cm^2/m^2 met the criteria for SAS, but adult dogs with EOAi between 1.29 and 2.3 cm^2/m^2 could not be classified on the basis of EOAi alone. Seven of 16 (44%) dogs with SAS (EOAi < 1.29 cm^2/m^2) and 10 of 22 (45%) unaffected dogs (EOAi > 2.3 cm^2/m^2, respectively) could be classified accurately based on EOAi alone. The remaining 9 dogs with SAS and 12 unaffected dogs would have been classified as equivocal on the basis of EOAi alone.

These results emphasize the relevance of EOAi more as a specific criterion to confirm SAS on the basis of different cutoffs based on the animal's age at the time of evaluation. In this study, all Golden Retriever puppies 2 to 6 months with an EOAi < 1.46 cm^2/m^2 and all adults after 12 months with an EOAi < 1.29 cm^2/m^2 were ultimately determined to have SAS.

For animals with equivocal EOAi, consideration of EOAi and LVOT Vmax together could add value to the

Table 2—Sensitivity, specificity, PPV, and NPV of combined EOAi and LVOT Vmax measured at baseline in 16 dogs that went on to satisfy the criteria for SAS at follow-up, compared with 22 unaffected dogs.

Diagnostic criteria	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Vmax > 2.3 m/s	63	100	100	79
EOAi < 1.46 cm^2/m^2	50	100	100	73
Vmax > 2.3 m/s or EOAi < 1.46 cm^2/m^2	69	100	100	81

diagnostic assessment. The maximal NPV obtained for EOAi in puppies occurred at 2.49 cm^2/m^2. Interestingly, a recent analysis on 1,283 Boxers demonstrated that the risk for SAS was 3 times as great for dogs with an aortic annulus area < 2.1 cm^2/m^2, compared with dogs with an aortic annulus area > 2.37 cm^2/m^2.[24] Our results are consistent with such findings and suggest that, when combined with LVOT Vmax, EOAi can provide additional useful information that may predict the development of SAS in some Golden Retrievers as puppies. Assessment of the EOAi conceivably could have particular importance in cases with left ventricular systolic dysfunction, cardiac arrhythmias, or other aberrations (eg, anemia, polycythemia, gammopathies, leukemias, and other causes of hyperviscosity syndrome) that could alter LVOT flow.[14,25–29]

On the basis of the results of the present study, the future development of SAS may be suspected in Golden Retriever puppies with an LVOT Vmax > 2.3 m/s; all puppies with an LVOT Vmax higher than this value ultimately met the criteria for SAS at adulthood. Conversely, these data suggest that Golden Retriever puppies with an LVOT Vmax < 1.57 m/s are highly unlikely to develop SAS at age 12 to 18 months. Predicting whether SAS will develop in Golden Retriever puppies with an LVOT Vmax between 1.58 and 2.3 m/s can be helped in some cases by the consideration of EOAi. Among the 16 dogs with SAS in this study, 6 (38%) would have been classified as unaffected or equivocal as puppies on the basis of their LVOT Vmax (range, 1.58 to 2.08 m/s). However, all puppies with an EOAi < 1.46 cm^2/m^2 developed SAS as adults, and 1 study dog with a normal baseline LVOT Vmax (1.96 m/s) had a low baseline EOAi (1.26 cm^2/m^2) and was ultimately classified as having SAS at follow-up (adult LVOT Vmax, 2.65 m/s; adult EOAi, 1.03 cm^2/m^2).

As expected, LVOT Vmax was higher in adult dogs with SAS, compared with LVOT Vmax recorded during puppyhood. This trend has already been observed in other breeds, including Boxers[7] and Newfoundlands,[8] and can be explained by the onset or progression of SAS during a puppy's growth. However, in Boxers, both unaffected and affected dogs have significantly higher LVOT Vmax as adults, and a breed-specific characteristic has been suspected.[7] In the present study of Golden Retrievers, there was a significant increase in LVOT Vmax from baseline to follow-up in both unaffected dogs ($P < 0.001$) and dogs that met the criteria for SAS ($P = 0.04$). Possible explanations include physiologic cardiac remodeling in growing patients with underlying changes in heart volume-to-mass ratio, effect of growth hormone impregnation on contractile proteins and myocyte hypertrophy, and changes in cardiac cell populations with the critical role of cardiac fibroblast cells in heart growth.[30,31] These findings identify that age may be a confounding factor when evaluating Golden Retrievers for SAS on the basis of LVOT Vmax alone. A change from baseline to follow-up was not observed with EOAi. In humans, it is accepted that aortic valve area may over- or underestimate the severity of aortic stenosis in patients with small or large body surface area.[11,13,32] Indexing EOA to body surface area is thus considered helpful in very small or large patients, reducing the effect of variable body size. This may also explain why EOAi was not significantly different between puppies and adults in our results.

Although widely used in the diagnosis of SAS in dogs, LVOT Vmax has imperfect sensitivity and specificity: dogs with normal Vmax may have SAS,[19] and dogs without SAS may have abnormal Vmax.[33] Yet LVOT Vmax remains the most practical antemortem diagnostic test for SAS in dogs, with a controversial cutoff variously reported between 2.0 and 2.3 m/s.[6,34,35] Unfortunately, the subtle nature of some SAS lesions and their effect on LVOT hemodynamics probably contribute substantially to the limitations of measurement of LVOT Vmax as a diagnostic test for SAS. Imperfect specificity is of great concern for selective breeding: individuals with SAS should not be missed when culling dogs from a breeding pool. Conversely, elimination of dogs with false-positive results can eventually lead to a restricted gene pool in the breed. A combination of screening variables might therefore prove useful to increase the accuracy of screening programs.

The combination of LVOT Vmax and EOAi yielded slightly higher sensitivity (69%) for SAS screening during puppyhood than Vmax or EOAi alone (63% and 50%, respectively). In humans, Doppler echocardiographic parameters generally used for identifying severity of aortic stenosis include LVOT Vmax, mean left-ventricular-to-aortic pressure gradient, and EOA because these are known to have important limitations if used in isolation.[26] Because flow may vary widely even if left ventricular function appears normal, calculation of EOA is considered essential.[11] None of the puppies included in this study developed evidence of low-flow severe SAS as may occur with left ventricular systolic dysfunction, which could eventually increase the usefulness of EOAi.

Aortic insufficiency is a common finding in Golden Retrievers with SAS, and several causative factors have been postulated to explain its frequent occurrence.[36] Turbulent flow jet damaging the valvular cusps and early systolic closure of the aortic valve have been suggested. As observed in children, AI was mild in most puppies evaluated in the present study, and the severity of AI increased significantly at follow-up in dogs with SAS.[37,38]

This study has inherent limitations. First, there is no antemortem gold standard for the diagnosis of mild or moderate SAS in dogs at the present time. Second, there is no widely accepted consensus on what should be accepted as the upper limit of Doppler-derived LVOT Vmax in the evaluation of dogs for SAS. Acceptance of breed-specific limits[7,24,33,39] is probably necessary and, even so, likely incomplete. We set the LVOT Vmax at 2.3 m/s as recommended by the European Society of Veterinary Cardiology.[6] The specificity of LVOT Vmax ≥ 2.3 m/s as a gold standard of diagnosis is imperfect, and unaffected individuals may have been included in the group of dogs with SAS if they had high-velocity LVOT flow and an associated murmur, in the absence of SAS. Conversely, dogs with very mild SAS and hemodynamic loading conditions that lowered LVOT Vmax and dogs that were genotypically abnormal but phenotypically normal could have been misclassified as unaffected. A third limitation was small sample size. Significance was

reached for several important variables, but meaningful subgroup analysis was not possible with this study population.

Aortic insufficiency may influence calculated EOAi. Moderate or severe AI can alter the calculated EOAi by affecting the continuity equation.[13,18] When excluding EOAi from dogs with moderate AI, we found that EOAi was still significantly lower in dogs with SAS than in unaffected dogs, both at baseline ($P = 0.01$) and follow-up ($P < 0.001$). The EOAi also did not change significantly ($P = 0.21$) from baseline to follow-up evaluation in both groups. Consequently, moderate AI did not appear to significantly affect the results of the present study.

Although echocardiograms were reviewed at a later time by a cardiologist, examiners who acquired the echocardiographic images were not blinded to previous auscultation or baseline examination at the time of follow-up because the physical examinations and echocardiograms were performed on the same day; this could have introduced bias. Also, intra- and interoperator variability was not evaluated in this study. However, intra- and interobserver variability of EOAi measurements have been reported to be low in human patients with aortic valve disease.[40]

The high prevalence of SAS (42%) in this population of overtly healthy dogs is surprising, and there are at least 3 factors that may explain this finding. First, SAS is overrepresented in Golden Retrievers.[2] Second, bias may have been introduced through the recruitment process. We have built a trusting relationship with area breeders; the assurance of medical confidentiality and the awareness by breeders of this study's focus on SAS may have encouraged some breeders to request evaluation for litters of puppies with possible risk factors for SAS (eg, relative known to have SAS). Third, the specificity of LVOT Vmax ≥ 2.3 m/s as a gold standard of diagnosis is imperfect, and unaffected individuals may have been included in dogs with SAS.

In conclusion, both LVOT Vmax > 2.3 m/s and EOAi < 1.46 cm^2/m^2 when measured in 2- to 6-month-old Golden Retriever puppies can be associated with a high likelihood that the same dogs as adults will satisfy the criteria for SAS. The combination of both criteria may offer a slightly higher sensitivity.

a. Model 811-B, Parks Medical Electronics Inc, Aloha, Ore.
b. Vivid-7 ultrasound system, GE Medical, Wauwatosa, Wis.
c. SAS, version 9.3, SAS Institute Inc, Cary, NC.

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