

Clinical Efficacy of Sildenafil in Treatment of Pulmonary Arterial Hypertension in Dogs

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Background: Pulmonary arterial hypertension (PAH) in dogs carries a poor prognosis. Sildenafil increases exercise capacity and improves hemodynamics in people with PAH.

Hypothesis/Objectives: Dogs receiving sildenafil will have lower pulmonary arterial pressure, increased exercise capacity, and better quality of life (QOL) than dogs receiving placebo.

Animals: Thirteen dogs with echocardiographic evidence of PAH.

Methods: Prospective short-term, randomized, placebo controlled, double-blind, crossover study. Dogs with PAH were randomly allocated to receive sildenafil or placebo for 4 weeks, followed by the alternative treatment for 4 weeks.

Results: Dogs receiving sildenafil had a significantly lower estimated pulmonary arterial pressure (median, 56 mmHg; range, 34–83 mmHg) than at baseline (median, 72 mmHg; range, 61–86 mmHg; $P = .018$), but not significantly lower than those receiving placebo (median, 62 mmHg; range, 49–197 mmHg). Exercise capacity was significantly greater in dogs receiving sildenafil than those receiving placebo (mean activity count per minute: 101 ± 47 versus 74 ± 32 ; $P = .05$). QOL scores were significantly higher in dogs receiving sildenafil than dogs receiving placebo.

Conclusions and Clinical Importance: Sildenafil decreases systolic pulmonary arterial pressure from baseline in dogs with PAH and is associated with increased exercise capacity and QOL when compared to treatment with placebo.

Key words: Echocardiography; Heart disease; Hemodynamics; Pulmonary disease.

Pulmonary arterial hypertension (PAH) is an uncommon condition characterized by an increase in pulmonary vascular resistance leading to right ventricular failure and premature death.¹ The prognosis in both people and dogs with PAH is poor, with median survival times of 3–91 days from diagnosis in dogs.^{2,3,4}

Cited treatment options for dogs with PAH include calcium channel blockers, diuretics, angiotensin converting enzyme inhibitors, digoxin, and thromboprophylaxis, but are often ineffective in dogs with severe PAH.^{2,4}

Drugs approved for people with PAH include intravenous, subcutaneous, and inhaled administration of prostacyclin analogues, the oral endothelin antagonist bosentan, and now sildenafil.⁵

Sildenafil is a selective inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase-5 that has been shown to improve exercise capacity, quality of life (QOL), and hemodynamics in human patients with symptomatic PAH.^{6–10} It enhances nitric oxide mediated pulmonary vasodilatation and is believed to have additional beneficial effects on vascular remodeling and cardiac function.^{11,12} Two retrospective studies of the use of sildenafil in dogs with pulmonary hypertension

Abbreviations:

6MWT	6-minute walk test
CVD	chronic valvular disease
PAH	pulmonary arterial hypertension
PG	systolic pressure gradient
QOL	quality of life
SABP	systemic arterial blood pressure

indicate that sildenafil has potential therapeutic efficacy.^{4,13} The aim of this study is to prospectively evaluate the effect of sildenafil on pulmonary arterial pressure, exercise capacity, and QOL (as assessed by owners) in dogs with PAH.

Methods

This was a randomized, placebo controlled, double-blind, crossover study. Dogs with PAH were eligible for enrollment after informed client consent had been obtained. Echocardiography was performed by a board certified or board eligible cardiologist (E.D. or M.M.S.) using a Sonos 7500 (Philips Medical Imaging; Andover, MA). A complete echocardiogram was obtained at each time point, including 2D, M-mode, and Doppler echocardiography. The tricuspid valve was imaged in the short and long axis views as well as obliquely to find the optimal (most parallel to the transducer beam) signal for Doppler measurement. The systolic pressure gradient (PGs) was estimated using the modified Bernoulli equation ($PGs = 4V^2$ where V is the maximal flow velocity across the tricuspid valve). Dogs were included if their PGs was at least 60 mmHg. The pulmonic valve was also interrogated to be certain that there was no evidence of pulmonic stenosis. Dogs were excluded if they had concurrent radiographic evidence of pulmonary edema consistent with left-sided congestive heart failure, or they had systemic hypertension/hypotension (> 180 mmHg or < 100 mmHg as measured by Doppler) or any unrelated severe comorbid condition. While changes in furosemide therapy made before the study were permitted, dogs were excluded if angiotensin converting enzyme inhibitors or phosphodiesterase inhibitors had been added to the

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treatment regimens within the prior 4 weeks. This study was approved by the Animal Care and Use Committee at the University of Pennsylvania.

Dogs were evaluated 3 times during the study: a baseline evaluation at enrollment (0 weeks), a 2nd evaluation after the 1st treatment phase (4 weeks), and a 3rd evaluation after the 2nd treatment phase (8 weeks). At the baseline evaluation the following were performed: echocardiogram, thoracic radiographs, arterial blood gas analysis^a (including electrolytes, lactate concentration, and hematocrit) and systemic arterial blood pressure (SABP; as measured by Doppler). A watch-sized activity monitor^b was placed on a collar and positioned ventrally on the neck of each dog for the duration of the study. The monitor continuously measured the intensity, frequency, and duration of movement by generation of a voltage from a piezoelectric sensor that is then converted to a raw activity value. Data was downloaded from the monitor after each phase of the study and converted to an activity count per minute using associated computer software. A detailed description of this device can be found elsewhere.^{14,15} QOL was assessed with a questionnaire (QOL1) at each of the 3 patient evaluations. QOL1 had just 1 question that was designed to determine the QOL for the dog during the preceding 2 weeks. There were 5 possible scores ranging from 1 (poor) to 5 (excellent). QOL2 was a 2nd questionnaire that was used for the 2nd and 3rd evaluation. QOL2 was designed to determine improvement or worsening of condition during the study. Similarly, the 7 possible scores ranged from 1 (much worse) to 7 (much better). The numerical scores from QOL1 and QOL2 were used in statistical analysis.

Study randomization and drug formulation was performed by the Investigational Drug Service of the Hospital of the University of Pennsylvania. Dogs were randomized to receive either sildenafil^c first or placebo first. Sildenafil was dispensed at a dose of 1 mg/kg every 8 hours PO and formulated into 1 mg (red), 2 mg (green), or 5 mg (blue) capsules. Placebo was dispensed using the same number and color of capsules. The dogs received the placebo (Phase P) or sildenafil (Phase S) for 4 weeks followed by the 2nd evaluation and study crossover after a 3-day wash out period. The 3rd evaluation was at 8 weeks, following both phases of the study.

At the 2nd and 3rd evaluations, echocardiography, arterial blood gas analysis, and SABP (Doppler) were measured. Data from the activity monitor were downloaded to a computer and the device reattached to the dog. QOL1 was given to the owner to complete, with QOL2 given 1 hour later.

Dogs were withdrawn from the study at the owner's request, if SABP decreased below 100 mmHg, or if the dog's condition deteriorated with a potential risk of mortality.

The intention-to-treat principle was applied in the data analysis. PAP_s, SABP, heart rate, arterial blood gas analysis, and QOL1 were analyzed for those patients that completed both phases of the study. Activity monitor data were analyzed on all dogs that entered the 2nd phase of the study. A QOL2 score of 1 was assigned to the phase during which an animal died or was euthanized due to worsening clinical signs.

All continuous variables were visually inspected and evaluated for normality using the D'Agostino and Pearson's omnibus normality test. Nonparametric data are reported as the median (range) and parametric data are reported as mean \pm standard deviation. Parametric data were analyzed using a paired *t*-test (when comparing Phase P to Phase S) or analysis of variance with repeated measures (when comparing Phases P and S to baseline), plus period as a factor. Nonparametric data were analyzed with the Wilcoxon signed rank test (when comparing Phase P to Phase S) or the Friedman test (when comparing Phases P and S to baseline). Post hoc analysis was performed using Tukey's multiple comparison test (parametric) or Dunn's multiple comparison test (nonparametric). Withdrawal from the study was compared between phases using Fisher's Exact. A value of $P < .05$ (two-sided) was considered

significant. All data analyses were performed using commercially available statistical software.^d

Results

Baseline Characteristics

Thirteen dogs were enrolled in the study. Median age was 11 years (range 6–13) and median weight was 8 kg (range 4–30). Eleven dogs were male (87%; all castrated) and 2 dogs were female (13%; 1 spayed and 1 intact). The study population consisted of 3 Chihuahuas, 2 Cocker Spaniels, 2 Dachshunds, and 1 each of mixbreed, Dalmatian, Jack Russell Terrier, Pomeranian, Shih Tzu, and Lhasa Apso. In all study dogs, severe PAH was present in conjunction with chronic valvular disease (CVD), and no underlying primary airway disease was identified with thoracic radiographs. However, further diagnostics to identify the presence of primary airway disease were not performed.

Medications administered during the study included furosemide (12), enalapril (11), pimobendan (5), digoxin (5), spironolactone (2), aldactazide (2), deracoxib (1), hydrocodone (1), and lysodren (1).

Six dogs were randomized to receive sildenafil-first, and 7 were randomized to receive placebo-first. There was no significant difference in age, QOL1, or PGs gradient at baseline between the sildenafil-first group and the placebo-first group (Table 1).

Eight dogs completed both phases of the clinical trial; 2 dogs were withdrawn from the 1st phase, and 3 dogs were withdrawn from the 2nd phase of the trial. All 5 of these dogs were withdrawn from the placebo phase (Phase P). Dogs were more likely to be withdrawn during Phase P than during Phase S ($P = .04$; $n = 13$). Mean baseline PGs in dogs completing the study was 69 ± 9 mmHg ($n = 8$), and 78 ± 8 mmHg in dogs withdrawn from the study ($P = .14$; $n = 5$). No adverse effects were reported in either phase.

Hemodynamics

Eight dogs had PGs measured at 3 time-points (baseline, after Phase S, and after Phase P). Median PGs was 72 (61–86) mmHg at baseline, 56 (34–83) mmHg after Phase S, and 62 (49–197) mmHg after Phase P. PGs after Phase S was significantly lower than baseline ($P = .018$; $n = 8$) but not significantly different from Phase P (Fig 1).

Table 1. Baseline characteristics of the study population.

	Sildenafil First (n = 6)	Placebo First (n = 7)
Age (years)	10 (9–14)	11 (6–13)
Male	6 (100%)	5 (71%)
Female	0 (0%)	2 (29%)
PGs (mmHg)	70 (61–84)	74 (71–87)
QOL1 score (1–5)	3 (2–5)	3 (2–5)
Weight (kg)	6.5 (4–15)	9 (5–30)

Nonparametric continuous data are presented as median (range), and categorical data are presented as number (% of total). There was no significant difference between dogs receiving sildenafil first and those receiving placebo first.

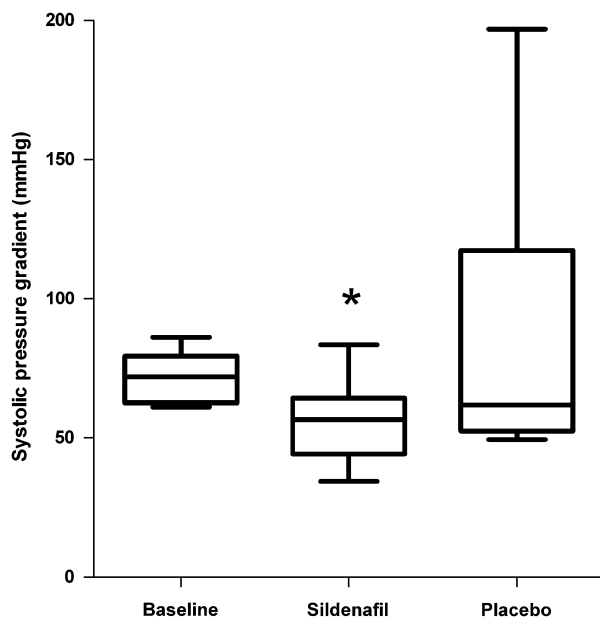


Fig 1. Box plots depicting systolic pressure gradient (PGs) in dogs with pulmonary arterial hypertension. There was a significant reduction in PGs after 4 weeks of sildenafil ($*P < .02$) but not after 4 weeks of placebo. For each plot, the central horizontal line is the median, the box represents the 25th–75th percentiles, and the whiskers represent the range.

There was no significant difference in heart rate or SABP between baseline, Phase S, or Phase P. Mean heart rate per minute was 147 ± 45 at baseline, 129 ± 31 following Phase S, and 129 ± 34 following Phase P ($n = 8$). Median SABP was 138 (95–165) mmHg at baseline, 126 (100–180) mmHg following Phase S, and 130 (87–170) mmHg following Phase P ($n = 6$).

Clinicopathologic Data

There was no significant difference in hematocrit, PaO₂, PaCO₂, electrolytes, or lactate concentration between phases (Table 2).

Activity and QOL

Activity was significantly greater during Phase S than during Phase P. Mean activity count per minute was 101 ± 47 during Phase S and 74 ± 32 during Phase P ($P = .05$; $n = 10$). Activity was significantly less during the 2nd phase of the study, irrespective of which treatment they received first ($P = .01$; $n = 10$; Fig 2).

QOL scores (QOL1 and QOL2) as assessed by owners were significantly higher at the end of Phase S than at the end of Phase P. QOL1 was 3.00 ± 1.1 at baseline, 3.50 ± 0.8 after Phase S, and 2.88 ± 1.0 following Phase P ($P = .04$; $n = 8$). Improvement in the QOL score (QOL2) was also significantly different between phases. Median QOL2 was 5 (range: 4–7) following Phase S, and 3 (range: 1–6) following Phase P ($P = .04$; $n = 9$).

Discussion

In this single-center, randomized, double-blind, placebo-controlled cross-over study, sildenafil significantly decreased pulmonary artery pressure gradient from baseline, and when compared with placebo, increased activity and QOL of dogs with PAH. Although this study was not designed to detect a difference in survival, it is of note that dogs were significantly more likely to be withdrawn from the study during Phase P than during Phase S. Four of these 5 dogs were withdrawn due to deteriorating clinical signs, and 1 was withdrawn due to family circumstances. There were no reported adverse effects of dogs receiving placebo or sildenafil in this study.

Sildenafil reduces pulmonary arterial pressure in people with PAH.^{6,7,16,17} In dogs, PGs significantly decreased after sildenafil therapy in 1 retrospective study,⁴ but was not associated with a significant decrease in peak tricuspid regurgitation flow gradient in a 2nd retrospective study.¹³ The PGs was significantly lower following 4 weeks of sildenafil than at baseline, although there was no significant difference in PGs after 4 weeks of sildenafil compared with 4 weeks of placebo in the current study. However, 5 dogs were withdrawn from the study and therefore only 8 dogs had PGs obtained at baseline and at the end of each phase of the study. It

Table 2. Comparison of selected hemodynamic and clinicopathologic results of dogs at baseline, and after 4 weeks of sildenafil or placebo.

	n	Baseline	Phase S (Sildenafil)	Phase P (Placebo)	P-Value
Heart rate (beats per minute)	8	147 ± 45	129 ± 31	129 ± 34	.137
SABP (mmHg)	6	138 (95–165)	126 (100–180)	130 (87–170)	.956
PGs (mmHg)	8	72 (61–86)	56 (34–83)	62 (49–197)	.018*
Hematocrit (%)	5	43 (38–48)	43 (37–50)	46 (35–52)	.691
PaO ₂ (mmHg)	7	66 (51–90)	61 (50–86)	61 (54–89)	.964
PaCO ₂ (mmHg)	5	36 (31–48)	32 (30–37)	34 (26–37)	.522
Alveolar-arterial gradient (A-a gradient; mmHg)	5	31 (11–55)	44 (21–60)	50 (22–58)	.953
Lactate concentration (mmol/L)	4	1.75 (0.9–2.7)	1.5 (0.3–2.8)	1.7 (1.3–3.3)	.933
Sodium (mmol/L)	6	145 (133–148)	144 (128–149)	142 (133–148)	.252
Potassium (mmol/L)	7	4.2 (3.5–4.8)	3.7 (3.0–5.0)	4.1 (3.4–5.5)	.486
Ionized Calcium (mmol/L)	5	1.24 (1.07–1.33)	1.22 (1.1–1.23)	1.23 (1.04–1.3)	.954

Nonparametric data are presented as median (range) and parametric data are presented as mean \pm standard deviation.

* $P < .05$ significant.

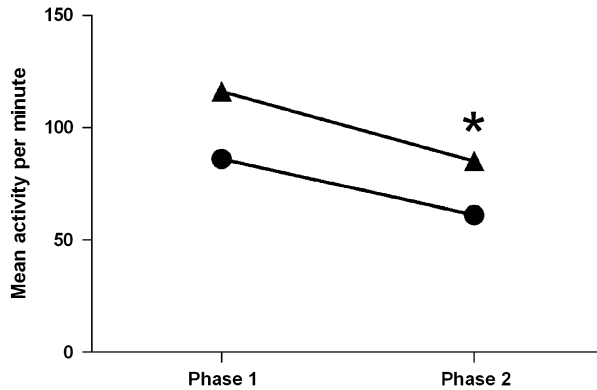


Fig 2. Graph depicting mean activity count of dogs with pulmonary arterial hypertension receiving sildenafil or placebo. Closed triangles represent sildenafil, and closed circles represent placebo. Mean activity is significantly less in Phase 2 (weeks 4–8) of the study than Phase 1 (weeks 1–4), irrespective of drug received (* $P = .01$).

is possible the loss of these measurements skewed our results due to a type II error.

Sildenafil is a selective inhibitor of phosphodiesterase-5 that causes pulmonary artery vasodilatation. Because of the high degree of localization of the phosphodiesterase-5 to the pulmonary vascular smooth muscle, a decrease in systemic blood pressure is not typically expected.^{7,18} However, in 1 retrospective study of dogs with PAH receiving sildenafil, a decrease (albeit statistically nonsignificant) in median systemic blood pressure of 33 mmHg was reported.⁴ In this current study, mean SABP (as measured by Doppler) did not significantly decrease from baseline in the dogs receiving sildenafil or placebo.

Exercise intolerance is common in dogs with PAH,² and the 6-minute walk test (6MWT) is an independent predictor of death in people with idiopathic PAH.¹⁹ Primary end points of many clinical trials in people with PAH have addressed exercise capacity, typically by using the (6MWT).¹⁰ Sildenafil has been shown to increase the distance walked in 6 minutes in a randomized double-blinded placebo controlled trial of 278 people with PAH.⁶ Although the 6MWT has been evaluated in dogs,²⁰ the severity of clinical signs afflicting these dogs with PAH prompted us to consider a different means of assessing exercise capacity. In this study we assessed activity in dogs by means of an accelerometer. Dogs had significantly greater activity during Phase S than during Phase P. This study was not designed to investigate the long-term effects of sildenafil on exercise tolerance. However, 1 extended study in people suggested that the effect of sildenafil monotherapy on exercise capacity is maintained after 1 year of treatment.⁶ The effect of treatment order on activity was also evaluated. Activity decreased in the 2nd phase of the cross over study irrespective of the treatment that dogs were receiving. This finding suggests that severe PAH is progressive and demonstrates that exercise tolerance is likely to deteriorate over time regardless of therapy.

Health-related quality of life (HRQoL) questionnaires have been used to assess the effect of sildenafil in people

with PAH.^{8,9} Although a questionnaire evaluating HRQoL of dogs with cardiac disease has been described, this current study was designed and cases were enrolled before this publication.²¹ We devised 2 simple questions to evaluate an owner's perception of the QOL of their dog. Owners assessed their dog's QOL score (QOL1) to be significantly better during Phase S than during Phase P, but not significantly better than at baseline. In addition, when owners were asked about the *change* in QOL of their dog, they believed that the QOL score (QOL2) was significantly better during Phase S than during Phase P.

There are several limitations to this study. The first is the small number of dogs enrolled. The second is the large number of dogs that were withdrawn from the study during the placebo phase. Not surprisingly, dogs with more severe disease were more likely to be withdrawn from the study. Although we expected deterioration in clinical signs during the placebo phase, we did not envision 5 of 13 dogs being withdrawn. This high rate of withdrawal made it difficult to interpret hemodynamic data given the small sample size that completed both phases of the trial. A 3rd limitation is the large number of and variation in medications the dogs were concurrently receiving. In order to minimize this effect, angiotensin converting enzyme inhibitors and phosphodiesterase inhibitors were not added to the treatment regimens 4 weeks prior to or during the study, with the aim of reducing the effect of these vasodilators on the study dogs. A recent study has evaluated the use of pimobendan in dogs with PAH secondary to degenerative mitral valve disease.²² The authors concluded that pimobendan reduced the severity of measurable PAH and improved QOL scores. It is of note that in this current study, 5 of 13 dogs were already receiving pimobendan before enrollment. This suggests that although pimobendan may ameliorate PAH in some dogs, the addition of sildenafil may provide further benefit. A final limitation developed over the course of the study as it became clear that many dogs improved during one phase of the study. This clinical finding made it challenging to enroll patients with severe PAH in a blinded placebo-controlled study.

In conclusion, this study demonstrates that sildenafil decreases PGs, increases exercise capacity, and improves QOL in dogs with PAH and underlying CVD. In addition, dogs were significantly less likely to be withdrawn from this blinded study while receiving sildenafil, than while receiving placebo.

Footnotes

^aNOVA Statlabs Profile 16, Biomedical, Waltham, MA

^bActical, Mini Mitter Inc, Bend, OR

^cViagra, Pfizer US Pharmaceuticals, New York, NY

^dPrism 5 for Windows, GraphPad Software Inc, San Diego, CA

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References

1. Rubin LJ. Primary pulmonary hypertension. *N Engl J Med* 1997;336:111–117.
2. Johnson L, Boon J, Orton EC. Clinical characteristics of 53 dogs with Doppler-derived evidence of pulmonary hypertension: 1992–1996. *J Vet Intern Med* 1999;13:440–447.
3. D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med* 1991;115:343–349.
4. Bach JF, Rozanski EA, MacGregor J, et al. Retrospective evaluation of sildenafil citrate as a therapy for pulmonary hypertension in dogs. *J Vet Intern Med* 2006;20:1132–1135.
5. Galie N, Seeger W, Naeije R, et al. Comparative analysis of clinical trials and evidence-based treatment algorithm in pulmonary arterial hypertension. *J Am Coll Cardiol* 2004;43:81S–88S.
6. Galie N, Ghofrani HA, Torbicki A, et al. Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med* 2005;353:2148–2157.
7. Michelakis E, Tymchak W, Lien D, et al. Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: Comparison with inhaled nitric oxide. *Circulation* 2002;105:2398–2403.
8. Pepke-Zaba J, Gilbert C, Collings L, et al. Sildenafil improves health-related quality of life in patients with pulmonary arterial hypertension. *Chest* 2008;133:183–189.
9. Sastry BK, Narasimhan C, Reddy NK, et al. Clinical efficacy of sildenafil in primary pulmonary hypertension: A randomized, placebo-controlled, double-blind, crossover study. *J Am Coll Cardiol* 2004;43:1149–1153.
10. Suntharalingam J, Treacy CM, Doughty NJ, et al. Long-term use of sildenafil in inoperable chronic thromboembolic pulmonary hypertension. *Chest* 2008;134:229–236.
11. Takimoto E, Champion HC, Li M, et al. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med* 2005;11:214–222.
12. Wharton J, Strange JW, Moller GM, et al. Antiproliferative effects of phosphodiesterase type 5 inhibition in human pulmonary artery cells. *Am J Respir Crit Care Med* 2005;172:105–113.
13. Kellum HB, Stepien RL. Sildenafil citrate therapy in 22 dogs with pulmonary hypertension. *J Vet Intern Med* 2007;21:1258–1264.
14. Hansen BD, Lascelles BD, Keene BW, et al. Evaluation of an accelerometer for at-home monitoring of spontaneous activity in dogs. *Am J Vet Res* 2007;68:468–475.
15. Dow C, Michel KE, Love M, et al. Evaluation of optimal sampling interval for activity monitoring in companion dogs. *Am J Vet Res* 2009;70:444–448.
16. Simonneau G, Rubin LJ, Galie N, et al. Addition of sildenafil to long-term intravenous epoprostenol therapy in patients with pulmonary arterial hypertension: A randomized trial. *Ann Intern Med* 2008;149:521–530.
17. Ghofrani HA, Wiedemann R, Rose F, et al. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: A randomized controlled trial. *Lancet* 2002;360:895–900.
18. Lepore JJ, Maroo A, Bigatello LM, et al. Hemodynamic effects of sildenafil in patients with congestive heart failure and pulmonary hypertension: Combined administration with inhaled nitric oxide. *Chest* 2005;127:1647–1653.
19. Miyamoto S, Nagaya N, Satoh T, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 2000;161:487–492.
20. Boddy KN, Roche BM, Schwartz DS, et al. Evaluation of the six-minute walk test in dogs. *Am J Vet Res* 2004;65:311–313.
21. Freeman LM, Rush JE, Farabaugh AE, et al. Development and evaluation of a questionnaire for assessing health-related quality of life in dogs with cardiac disease. *J Am Vet Med Assoc* 2005;226:1864–1868.
22. Atkinson KJ, Fine DM, Thombs LA, et al. Evaluation of pimobendan and N-terminal probrain natriuretic peptide in the treatment of pulmonary hypertension secondary to degenerative mitral valve disease in dogs. *J Vet Intern Med* 2009;23:1190–1196.