Received: 19 August 2020 | Accepted: 19 November 2020

DOI: 10.1111/jvim.15978

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Changes in systolic blood pressure in dogs with pituitary dependent hyperadrenocorticism during the first year of trilostane treatment

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Funding information
Royal Canin

Abstract

Background: Systemic hypertension (SH) is common in dogs and humans with hypercortisolism and can persist after treatment.

Objectives: To evaluate changes in prevalence of SH and systolic blood pressure (SBP) in dogs with pituitary-dependent hyperadrenocorticism (PDH) during the first year of trilostane treatment, its relationship with disease control and selected laboratory variables, and their response to antihypertensive treatment.

Animals: Fifty-one dogs with PDH treated with trilostane Q12h.

Methods: Prospective case series study. Dogs were evaluated at diagnosis (T0) and 1, 3, 6, and 12 months (T12). Dogs were classified as nonhypertensive (SBP < 160 mm Hg) or hypertensive (SBP≥160 mm Hg) and subclassified according to target organ damage (TOD) risk. Hypertensive dogs were treated with benazepril and, if control of SH was not achieved, amlodipine was added.

Results: Prevalence of SH decreased from T0 (36/51) to T12 (17/37; P = .01). Changes in SBP during the study were influenced by the risk of TOD at T0. In severely hypertensive (SBP ≥ 180 mm Hg) dogs, the decrease in SBP was more pronounced whereas in normotensive (SBP < 140 mm Hg) dogs SBP increased slightly (P = .00). Blood pressure was not associated with disease control. Antihypertensive treatment was needed in 31/51 dogs, and in 13/31 dogs additional SH control with amlodipine was required. One third of nonhypertensive dogs at T0 required treatment with benazepril because SH developed during follow-up.

Conclusions and Clinical Importance: In dogs with PDH, SBP should be measured at every visit, regardless of disease control or SBP at diagnosis. More than 1 drug may be necessary to manage SH in affected dogs.

Abbreviations: 11β-HSD, 11β-hydroxysteroid dehydrogenase; ACEI, angiotensin converting enzyme inhibitor; ACTH-st, ACTH stimulation test; ARB, angiotensin II receptor blocker; BCS, body condition score; CD, Cushing's disease; CS, Cushing's syndrome; HAC, hyperadrenocorticism; HT, hypertensive; IQR, interquartile range; LDDST, low-dose dexamethasone suppression test; MC, moderately controlled; MR, mineralocorticoid receptor; NHT, nonhypertensive; PC, poorly controlled; PDH, pituitary-dependent hyperadrenocorticism; SBP, systolic blood pressure; SH, systemic hypertension; TOD, target organ damage.

KEYWORDS
angiotensin converting enzyme inhibitors, benazepril, canine, cortisol, Cushing's, hypertension

1 | INTRODUCTION

Hyperadrenocorticism (HAC) is a relatively common endocrine disease in dogs that results from chronic glucocorticoid excess with a reported prevalence in dogs of 0.2% to 0.28%,[1,2] higher than in people with Cushing's syndrome (CS).[3,4] Systemic hypertension (SH) is a frequent complication of this condition affecting 70-85% of people with CS[4-7] and 31% to 86% of dogs with HAC at diagnosis.[8-14] Systemic hypertension in affected people and dogs can persist even after appropriate control of the disease.[4-9]

In people with CS, systolic blood pressure (SBP) and prevalence of SH tend to decrease after surgical treatment of the disease but in 20% to 40% of patients, SH can persist after years of definitive treatment.[4-7,15-20] In people with CS treated with ketoconazole, metyrapone, pasireotide, mifepristone, or cabergoline, a decrease in blood pressure has been shown.[7,21-27] In children and adults with CS, increased arterial rigidity has been observed even after successful surgical treatment,[16,28,29] and in adults, increased cardiovascular risk and mortality rate still persist after successful treatment of the disease.[17,18,30,31] Thus, most investigators agree that normalization of cortisol concentrations does not always result in disappearance of the metabolic and vascular changes related to CS.[4-7]

In human medicine, a treatment algorithm for SH in patients with CS has been proposed, with angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) being the first line drugs, and calcium channel blockers being the second line drugs.[4-7] Because lower serum potassium concentrations have been found to be related to SH because of an apparent mineralocorticoid excess, treatment with a mineralocorticoid receptor (MR) antagonist is recommended if mineralocorticoid excess is suspected.[4-7,32,33] Most patients require >1 antihypertensive drug to manage SH.[4-7] Also, in dogs with HAC, serum potassium concentrations recently have been reported to be negatively correlated with SBP.[13]

In dogs with HAC, 3 studies have evaluated SBP before and after treatment. Two studies evaluated SBP in dogs with pituitary-dependent HAC (PDH) after 3 months of treatment with mitotane. A decrease in SBP was found only in those dogs in which appropriate control of PDH was achieved.[8,10] Only 1 study has evaluated changes in SBP in dogs with PDH during trilostane treatment or after hypophysectomy. One study did not find differences in SBP or in the prevalence of SH during the first year of treatment with trilostane or after hypophysectomy.[9] To our knowledge, response to antihypertensive treatment in dogs with PDH has not been evaluated.

Our aims were to evaluate changes in SBP and prevalence of SH in dogs with PDH treated with trilostane twice daily during the first year of treatment, the relationship of SH to disease control, CBC results, serum potassium and cortisol concentrations at every time point, and response to antihypertensive treatment.

2 | MATERIALS AND METHODS

Dogs newly diagnosed with PDH at the Veterinary Teaching Hospital Complutense of Madrid between January 2015 and December 2018 were prospectively included in the study. All owners consented the use of data from their pets for research purposes at admission.

Diagnosis of HAC was based on the presence of compatible clinical signs and physical examination findings. Definitive diagnosis was considered if ≥2 of the following tests were positive: urinary cortisol-to-creatinine ratio, ACTH stimulation test (ACTH-st), or low-dose dexamethasone suppression test (LDDST). Discrimination between adrenal dependent HAC and PDH was based on ultrasonography findings, plasma endogenous ACTH concentrations, and LDDST results.[34]

After definitive diagnosis of PDH, all dogs were treated with trilostane at a starting dosage of 0.3 to 1.0 mg/kg PO q12h. Dogs were evaluated at diagnosis (T0) and at 1, 3, 6, and 12 months after the beginning of trilostane treatment (T1, T3, T6, and T12, respectively). Exclusion criteria were the presence of chronic kidney disease of International Renal Interest Society (IRIS) stage 3 or 4, previous treatment with antihypertensive drugs or trilostane, and patients lost to follow-up on any of the visits. Death or development of hypoadrenocorticism during the study period was not an exclusion criterian.

Signalment, clinical signs reported by the owner, duration of clinical signs before diagnosis, physical examination findings, and presence of any concurrent disease were recorded at T0. Weight and body condition score (BCS) also were evaluated; dogs were classified as underweight (BCS ≤ 3/9), ideal body weight (BCS 4-6/9) or overweight (BCS ≥ 7/9).

At all evaluations, a complete clinical history and physical examination were performed, as well as an ACTH-st and serum electrolytes concentrations to monitor trilostane treatment. Additionally, at T0 and T12 a CBC was performed. During the study period, synthetic ACTH was not commercially available for 1 year and thus some ACTH-st results are missing. Cortisol concentrations were measured by chemiluminescence immunoassay (Immulite 2000, Siemens Healthcare S.L.U., Madrid, Spain).

For the purpose of the study, dogs were classified based on the owner´s opinion as, poorly controlled (PC), if clinical signs of HAC still were present; moderately controlled (MC), if evident improvement was observed but subtle clinical signs still were present, and well-controlled (WC) if the owners believed complete resolution of clinical signs was achieved.

At all visits, SBP was measured using Doppler ultrasonography (Vettex Uni 900, 65 Huntleigh Diagnostics Ltd, Cardiff, UK), with the dog in sternal or lateral recumbency with minimum restraint. An 8 MHz flat probe was placed between the carpal and metacarpal pad of the left forelimb. Cuff size was chosen to cover

30% to 40% of the limb circumference at the site of cuff placement.[35] Systolic blood pressure was recorded as the mean of at least 5 reliable measurements obtained after an acclimation period of approximately 5 minutes and before any other procedure was performed. Results of SBP ≥ 160 mm Hg were considered SH.[35] Dogs were classified as nonhypertensive (NHT, SBP < 160 mm Hg) or hypertensive (HT, SBP ≥ 160 mm Hg). Dogs also were subclassified according to the potential risk of target organ damage (TOD) following ACVIM guidelines as normotensive (SBP < 140 mm Hg), prehypertensive (SBP 140-159 mm Hg), moderately HT (SBP 160-179 mm Hg), and severely HT (SBP ≥ 180 mm Hg).[35] Additional visits to reevaluate SBP other than the previously described study visits (T1, T3, T6, and T12) were completed when necessary, following ACIM consensus recommendations to control SH.[35,36] Systolic blood pressure at T0 was recorded before any antihypertensive treatment was prescribed.

Antihypertensive treatment was prescribed based on ACVIM recommendations.[36] If signs of TOD were observed, antihypertensive treatment was started. If no signs of TOD were observed, dogs with SBP ≥160 mm Hg were reevaluated 7 days later. If SBP ≥180 mm Hg, hypertension was treated. If SBP was between 160 and 179 mm Hg, based on clinical judgment antihypertensive treatment either was prescribed or SBP was reevaluated 1 month later. Despite not being included in the guidelines, if SBP ≥200 mm Hg and no stressful events were recorded, antihypertensive treatment was started. Dogs diagnosed with SH initially were treated with benazepril at a starting dosage of 0.25 mg/kg PO q12h which was further increased to a maximum dosage of 0.5 mg/kg PO q12h if needed to control SBP. If control of SBP with benazepril was not achieved, amlodipine was added at a starting dosage of 0.1 to 0.25 mg/kg PO q24h; amlodipine dose was increased if necessary to a maximum dose of 0.5 mg/kg q24h. If control of SH still was not achieved, hydralazine was added (starting at a dosage of 0.2 mg/kg PO q12h). Evaluation of SBP was performed 15 days after any antihypertensive treatment was instituted. Additional reevaluations were scheduled every 15 days if SBP was >160 mm Hg. If SBP was <120 mm Hg with concurrent clinical signs of hypotension (eg, weakness, syncope, tachycardia) antihypertensive medication dose was decreased.[35,36] An ACEI was chosen as first line treatment based on a proposed treatment algorithm for people with CS[5] and also based on ACVIM guidelines.[35,36] However, as true for most ACEIs, benazepril must be hydrolyzed in the liver to its active metabolite. Because dogs with HAC tend to have increased liver enzymes activities, alanine aminotransferase (ALT) and alkaline phosphatase (ALKP) activity were evaluated at T0, T6, and T12.

Statistical analyses were performed using computer software (IBM, SPSS statistics for Windows, v.25.0, IBM Corp, Amornk, New York). Because most variables were not normally distributed, based on the Saphiro-Wilk test, nonparametric tests were performed. To compare categorical nonrepeated measures between groups, Fisher's exact test was used for dichotomical variables and Chi-squared tests were used for variables with >2 categories (data expressed as percentage). To compare data distribution of continuous nonrepeated measures between 2 groups, a Mann-Whitney *U* test was used, and between >2 groups, the Kruskal-Wallis test was used; results are expressed as median, range, and interquartile range (IQR). Correlation between continuous variables was evaluated using Spearman's rank correlation test. To compare categorical repeated measures among different endpoints, McNemar's test was used for dichotomical variables, and for those with >2 categories, Friedman test was used (results expressed as percentage). To compare continuous repeated measures, a Wilcoxon signed-rank test was used (results expressed as median, range, and IQR).

For dogs that completed the study period until T6 or until T12, assessment of changes in mean SBP results depending on the risk of TOD at T0 was performed using a repeated measurement analysis with a Greenhouse-Geisser correction (data expressed as mean ± SD).

3 | RESULTS

Fifty-one dogs with a definitive diagnosis of PDH were included. Fourteen dogs died during the study period; 4 before T3, 2 between T3 and T6, and 8 between T6 and T12. Causes of death were euthanasia because of poor quality of life based on the owner's opinion (5/14), neoplasia (eg, gastric carcinoma, prostatic carcinoma, pancreatic carcinoma, lymphoma; 4/14), complications related to HAC (eg, gallbladder mucocele, pituitary macroadenoma; 3/14), diabetic ketoacidosis (1/14), and unknown (sudden death; 1/14). Only 1 dog developed permanent hypoadrenocorticism at T6. This dog was treated initially with prednisone and fludrocortisone; the prednisone dose was tapered, and prednisone was discontinued after 15 days of treatment and the dog maintained long term using fludrocortisone.

3.1 | Signalment, clinical signs, physical examination findings, and concurrent diseases at T0

Median age was 11 years (range, 6-18 years; IQR, 10-13 years) and median weight was 11 kg (range, 1.9-40.8 kg; IQR, 6.8-16.5 kg). Nine dogs were intact females (9/51, 17.6%), 7 intact males (7/51, 13.7%), 24 spayed females (24/51, 47.1%), and 11 neutered males (11/51, 21.6%). Thirty-two (32/51, 62.7%) were pure breed dogs and 19/51 (37.3%) mixed breed dogs. Breeds represented were Yorkshire Terrier (5/51, 9.8%), West Highland White Terrier (3/51, 5.9%), French Bulldog (3/51, 5.9%), Maltese (3/51, 5.9%), Shih Tzu (2/51, 3.9%), Cocker Spaniel (2/51, 3.9%), Labrador Retriever (2/51, 3.9%), Beagle (2/51, 3.9%), and 1 of each of Boxer, Pit Bull, Miniature Poodle, Dalmatian, Lhasa Apso, and Dachshund.

At T0, 46/51 dogs (90.2%) had polydipsia, 44/51 (86.3%) polyuria, 46/51 (90.2%) polyphagia, and 30/51 (58.8%) had panting at rest. Neurological signs (eg, seizures, Horner's syndrome, vestibular disease, dullness, aggressiveness) were reported in 9/51 dogs (17.6%). Median duration of clinical signs before diagnosis was 6 months (range, 1-36 months; IQR, 4-12 months).

On physical examination, 39/51 dogs (76.47%) had coat abnormalities (eg, alopecia, dull coat) and 35/51 (68.6%) thin skin. Abdominal distension was present in 32/51 (62.7%) dogs. Only 3/51 (5.9%) had calcinosis cutis macroscopically. At T0, 3/51 dogs had a BCS <4

(5.9%), 25/51 (49%) had a BCS between 4 and 6 and 23/51 (45.1%) were overweight (BCS 7-9).

Concurrent diseases at T0 were: neoplasia (11/51, 21.6%), mitral valve disease (8/51, 15.9%), chronic kidney disease IRIS stage 2 (5/51, 9.8%), diabetes mellitus (5/51, 9.8%), leishmaniasis (2/51, 3.9%), hypothyroidism (2/51, 3.9%), pancreatitis (2/51, 3.9%), idiopathic epilepsy (1/51, 2.0%), and inflammatory bowel disease (1/51, 2.0%). Four dogs (4/51, 7.8%) were misdiagnosed previously with hypothyroidism and had been treated with thyroxine for 1 to 12 months before HAC diagnosis.

3.2 | Prevalence of SH and median SBP at the different endpoints

Fifteen dogs (15/51, 29.4%) were NHT (SBP < 160 mm Hg) at T0, and 36/51 dogs (70.6%) were considered HT (SBP ≥ 160 mm Hg). When subcategorized based on TOD, 9/51 dogs (17.6%) were normotensive, 6/51 dogs (11.8%) prehypertensive, 16/51 (31.4%) moderately hypertensive and 20/51 (39.2%) severely hypertensive. Median SBP at T0 was 170 mm Hg (range, 120-280 mm Hg; IQR, 150-190 mm Hg). Prevalence of SH and median SBP at the different endpoints, as well as significant differences among them are presented in Table 1.

3.3 | Relationship between BP at T0 and signalment, clinical signs, physical examination findings, and concurrent diseases

No differences were observed between prevalence of SH or median SBP and breed, sex, reproductive status, weight, clinical signs at diagnosis, duration of clinical signs, concurrent diseases, and physical examination findings.

Age at diagnosis was significantly correlated with SBP at T0 (rho = 0.355, P = .01); HT dogs were older (median, 12 years; range, 7-18 years; IQR, 11-13 years) than NHT dogs (median, 10 years; range, 6-13 years; IQR, 8.5-12 years; P = .02).

3.4 | Changes in mean SBP depending on risk of TOD at T0

Considering T6 as the first endpoint, 45 dogs were included. Changes in SBP between T0 to T6 were influenced by risk of TOD at T0 (P = .00). Of these dogs, 9/45 (20%) were normotensive at T0, 5/45 (11.1%) were prehypertensive, 15/45 (33.3%) moderately HT, and 16/45 (35.5%) severely HT. Mean results at every time point for each group are presented in Table 2 and graphical representation of mean SBP is shown in Figure 1.

Considering T12 as the second endpoint, 37 dogs were included. Changes in SBP during the study period T0 to T12 also were influenced by risk of TOD at T0 (P = .00). Of these dogs, 8/37 (21.6%) were normotensive, 4/37 (10.8%) prehypertensive, 10/37 (27%) moderately HT, and 15/37 (40.5%) severely HT at T0. Mean results at all time points for each group are presented in Table 3 and changes in mean SBP are shown in Figure 2.

3.5 | Relationship between blood pressure and control of disease based on clinical signs

At T1, 19/51 dogs (37.3%) were categorized as WC (complete resolution of clinical signs based on the owner's opinion), 34/47 (72.3%) at T3, 32/45 (71.1%) at T6, and 28/37 (75.7%) at T12. All dogs achieved WC, at 1 time point at least. No statistically significant differences were observed between prevalence of SH or median SBP and control

TABLE 1 Prevalence of systemic hypertension (SH), classification according to the risk of target organ damage (TOD) and median systolic blood pressure (SBP) values at the different endpoints. Superscript letters indicate differences statistically significant (a: P = .007, b: P = .05, c: P = .005, d: P = .004, e: P = .002, f: P = .003, g: P = .03, h: P = .003, i: P = .00)

	T0	T1	T3	T6	T12
Number of animals	51	51	47	45	37
SH (SBP ≥ 160 mm Hg)	36 (70.6%)[a,b]	29 (56.9%)	27 (57.4%)	18 (40%)[a]	17 (45.9%)[b]
Classification according to risk of TOD					
Normotension (SBP < 140 mm Hg)	9 (17.6%)	5 (9.8%)	4 (8.5%)	12 (26.7%)	11 (29.7%)
Prehypertension (SBP 140-159 mm Hg)	6 (11.8%)	17 (33.3%)	16 (34.0%)	15 (33.3%)	9 (24.3%)
Moderate hypertension (160-179 mm Hg)	16 (31.4%)	18 (35.3%)	13 (27.7%)	13 (28.9%)	13 (35.1%)
Severe hypertension (≥180 mm Hg)	20 (39.2%)[c]	11 (21.6%)	14 (29.8%)	5 (11.1%)[c]	4 (10.8%)[c]
SBP (mm Hg)					
Median	170[d,e]	160[f,g]	168[h,i]	151[d,f,h]	152[e,g,i]
Range (Minimum-Maximum)	120-280	110-280	107-244	116-220	101-210
IQR (Q1-Q3)	150-190	150-173	147-180	133-167	133-168

Abbreviations: IQR, interquartile range (expressed as quartile 1 [Q1] to quartile 3 [Q3]); T0, diagnosis; T1, T3, T6, and T12, 1, 3, 6, and 12 months after beginning of trilostane treatment, respectively.

TABLE 2 Mean systolic blood pressure (SBP) ± SD from T0 to T6 depending on the risk of target organ damage (TOD) at T0

	Mean systolic blood pressure (mean ± SD mm Hg)			
	T0	T1	T3	T6
Classification according to the risk of TOD at T0				
Normotensive (n = 9) (SBP < 140 mm Hg)	128 ± 6	140 ± 15	152 ± 16	147 ± 15
Prehypertensive (n = 5) (SBP 140-159 mm Hg)	150 ± 7	151 ± 7	147 ± 13	146 ± 20
Moderately hypertensive (n = 15) (SBP 160-179 mm Hg)	167 ± 6	160 ± 16	171 ± 26	155 ± 24
Severely hypertensive (n = 16) (SBP ≥ 180 mm Hg)	203 ± 30	183 ± 41	177 ± 39	155 ± 26

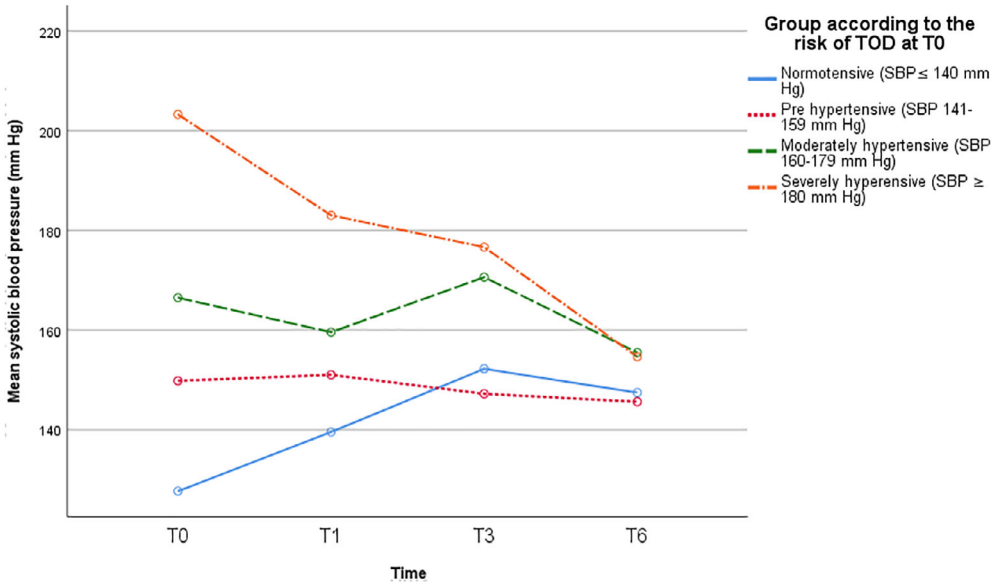

FIGURE 1 Evolution of mean systolic blood pressure (SBP) from T0 (diagnosis) to T6 (6 months after initiation of treatment) for the different groups of dogs classified at T0 according to the risk of target organ damage (TOD)

TABLE 3 Mean systolic blood pressure (SBP) ± SD from T0 to T12 depending on the risk of target organ damage (TOD) at T0

	Mean systolic blood pressure (mean ± SD mm Hg)				
	T0	T1	T3	T6	T12
Classification according to the risk of TOD at T0					
Normotensive (n = 8) (SBP < 140 mm Hg)	128 ± 7	138 ± 15	152 ± 17	148 ± 16	138 ± 22
Prehypertensive (n = 4) (SBP 140-159 mm Hg)	150 ± 8	151 ± 8	149 ± 14	141 ± 19	146 ± 23
Moderately hypertensive (n = 10) (SBP 160-179 mm Hg)	167 ± 6	161 ± 18	180 ± 26	162 ± 27	160 ± 21
Severely hypertensive (n = 15) (SBP ≥ 180 mm Hg)	205 ± 30	188 ± 37	181 ± 36	156 ± 26	157 ± 23

of the disease at any time point. Data on the prevalence of SH, number of dogs in each category of risk of TOD, and median SBP at every time point based on control of the disease are summarized in Table 4.

3.6 | Relationship between blood pressure and selected laboratory parameters

A CBC was performed at T0 and at T12. Results of the CBC were not significantly correlated with SBP. However, median platelet count was higher in HT dogs at T0 (median, 490.5 × 10^3/μL; range, 223-1115 × 10^3/μL; IQR, 410-575 × 10^3/μL) than in NHT dogs (median, 338 × 10^3/μL; range, 190-561 × 10^3/μL; IQR, 289-375 × 10^3/μL; P = .002), but not at T12.

Serum potassium concentrations, basal serum cortisol concentrations, and 1-hour post-ACTH serum cortisol concentrations were evaluated at every time point. However, because ACTH was not commercially available during part of the study period, basal serum cortisol concentrations were available in 49/51 dogs at T0, 49/51 dogs at T1, 45/47 dogs at T3, 44/45 dogs at T6, and 34/37 dogs at T12. One-hour post-ACTH serum cortisol concentrations were available in 43/51 dogs at T0, 47/51 dogs at T1, 45/47 dogs at T3, 41/45 dogs at T6, and 33/37 dogs at T12.

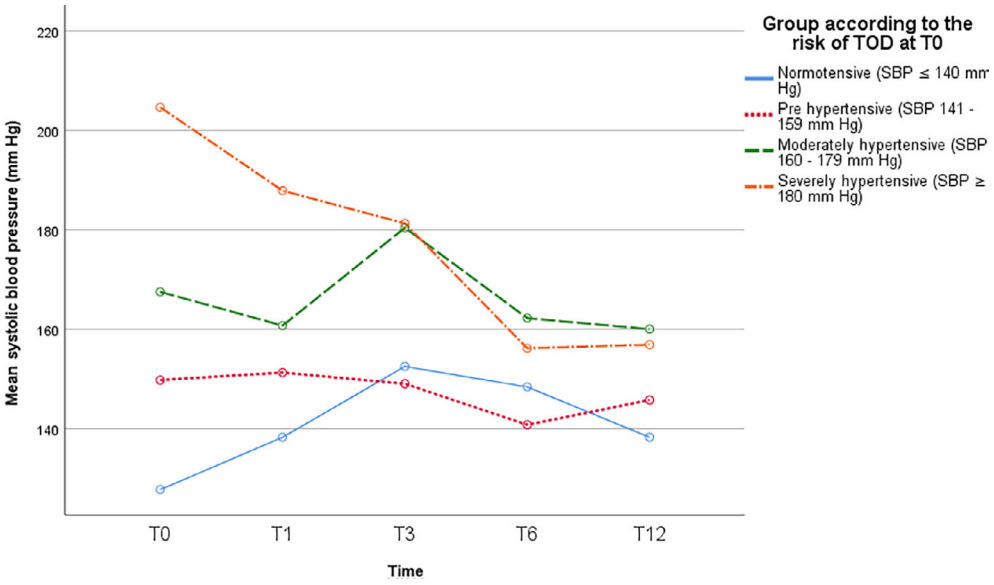

FIGURE 2 Evolution of mean systolic blood pressure (SBP) from T0 (diagnosis) to T12 (12 months after initiation of treatment) for the different groups of dogs classified at T0 according to the risk of target organ damage (TOD)

Serum potassium concentrations and SBP at T0 were negatively correlated (rho = –0.293, P = .04). Hypertensive dogs (T0) had lower median serum potassium concentrations (median, 4.0 mEq/L; range, 3.1-5.5 mEq/L; IQR, 3.9-4.3 mEq/L) than NHT dogs (median, 4.3 mEq/L; range, 3.5-5.3 mEq/L; IQR, 4.0-4.6 mEq/L; P = .05). Correlations were not found between serum potassium concentrations and SBP, and differences in median serum potassium concentrations were not found between NHT and HT at T1, T3, T6, and T12.

Basal serum cortisol concentrations and SBP were not significantly correlated at T0 and median basal serum cortisol concentrations were not significantly different between NHT and HT dogs. A significant correlation between SBP and basal serum cortisol concentrations was found at T1 (rho = 0.283, P = .05), T3 (rho = 0.406, P = .006), T6 (rho = 0.329, P = .03), and T12 (rho = 0.495, P = .003). Median basal serum cortisol concentrations at T6 were significantly higher in HT dogs (median, 3.23 μg/dL; range, 0.35-6.72 μg/dL; IQR, 2.37-5.7 μg/dL) than in NHT dogs (median, 2.02 μg/dL; range, 0.22-5.22 μg/dL; IQR, 1.32-2.99 μg/dL; P = .008). Similar changes also were found at T12 (median, 2.77 μg/dL; range, 1.21-7.31 μg/dL; IQR, 2.12-5.06 μg/dL for HT dogs and median, 1.23 μg/dL; range, 0.46-3.94 μg/dL; IQR, 0.87-2.8 μg/dL for NHT dogs; P = .001).

Serum cortisol concentrations 1-hour post-ACTH and SBP were correlated only at T12 (rho = 0.358, P = .04). Median 1-hour post-ACTH serum cortisol concentrations were significantly higher in HT dogs at T12 (median, 5.35 μg/dL; range, 2.97-18.70 μg/dL; IQR, 4.24-8.27 μg/dL) than in NHT dogs (median, 3.44 μg/dL; range, 1.09-6.56 μg/dL; IQR, 2.59-4.96 μg/dL; P = .002).

3.7 | Relationship between blood pressure and death during the study period

Neither median SBP at T0 nor prevalence of SH were significantly different between dogs that died during the study period (median, 166 mm Hg; range, 127-250 mm Hg; IQR, 160-200 mm Hg; 11/14, 78.6%) and survivors (median, 170 mm Hg; range, 120-280 mm Hg; IQR, 150-183 mm Hg; P = .81; 25/37, 67.6%; P = .51). No differences were found in the risk of TOD at T0 between survivors and non survivors. Mean SBP, prevalence of SH, and risk of TOD at T0 were not significantly different between dogs that died at different endpoints. Also, no differences were observed between dogs that died during the study and SBP, SH or risk of TOD at T1, T3, or T6. This evaluation was not made at T12 because all measures corresponded to dogs that completed the study.

3.8 | Antihypertensive treatment

Hypertension was treated according to ACVIM guidelines[35,36] in 31 dogs. Dogs that needed any antihypertensive treatment were more frequently HT at T0 (26/31, 83.9%; P = .01). Antihypertensive treatment was needed in 3/9 (33.3%) of the normotensive dogs at T0 and in 2/6 (33.3%) of the prehypertensive dogs at T0 because they developed SH during follow-up, as well as in 8/16 (50%) of the dogs with moderate hypertension, and in 18/20 (90%) of severely HT dogs at T0 (P = .005). Antihypertensive treatment was prescribed in 20/31 dogs (64.5%) at T0, in 3/31 (9.7%) at T1, in 4/31 (12.9%) at T3, in 3/31 (9.7%) at T6, and in 1/31 (3.2%) at T12. If antihypertensive treatment was prescribed between 2 endpoints, it was considered to have been started at the nearest endpoint.

During the study period, 31/51 dogs (60.8%) were treated with benazepril. In 13/31 dogs (41.9%), a second drug (ie, amlodipine) was added to control hypertension. Only 1 dog (1/31, 3.2%) needed a third drug (ie, hydralazine) to manage SH. All dogs that needed a second drug (13/13, 100%) were HT at T0, 6/13 (46.1%) being moderately HT and 7/13 (53.9%) being severely HT at diagnosis. The only dog that needed a third drug was severely HT at T0. Antihypertensive treatment could not be decreased in any dog.

Of the NHT dogs at T0, 9/15 (60%) had SBP≥160 mm Hg at least at 1 time point. In all cases, hypertension was moderate (SBP between

TABLE 4 Prevalence of systemic hypertension, classification according to the risk of target organ damage (TOD), and median systolic blood pressure (SBP) values at the different endpoints depending on the control of the disease

	T1 (n = 51)			T3 (n = 47)			T6 (n = 45)			T12 (n = 37)		
	PC (n = 12)	MC (n = 20)	WC (n = 19)	PC (n = 2)	MC (n = 11)	WC (n = 34)	PC (n = 4)	MC (n = 9)	WC (n = 32)	PC (n = 3)	MC (n = 6)	WC (n = 28)
Systemic Hypertension (SBP ≥ 160 mm Hg)	8 (66.7%)	12 (60%)	9 (47.4%)	0 (0%)	8 (72.7%)	19 (55.9%)	0 (0%)	5 (55.6%)	13 (40.6%)	1 (33.3%)	1 (16.7%)	15 (53.6%)
Classification according to the risk of TOD												
Normotension (SBP < 140 mm Hg)	1 (8.3%)	2 (10%)	2 (10.5%)	1 (50%)	1 (9.1%)	2 (5.9%)	0 (0%)	0 (0%)	12 (37.5%)	0 (0%)	4 (66.7%)	7 (25%)
Prehypertension (SBP 140-159 mm Hg)	3 (25%)	6 (30%)	8 (42.1%)	1 (50%)	2 (18.2%)	13 (38.2%)	4 (100%)	4 (44%)	7 (21.9%)	2 (66.6%)	1 (16.7%)	6 (21.4%)
Moderate hypertension (SBP 160-179 mm Hg)	3 (25%)	9 (45%)	6 (31.6%)	0 (0%)	5 (45.5%)	8 (23.5%)	0 (0%)	2 (22.2%)	11 (34.4%)	1 (33.3%)	1 (16.7%)	11 (39.3%)
Severe hypertension (SBP ≥ 180 mm Hg)	5 (41.7%)	3 (15%)	3 (15.8%)	0 (0%)	3 (27.3%)	11 (32.4%)	0 (0%)	3 (33.3%)	2 (6.3%)	0 (0%)	0 (0%)	4 (14.3%)
Systolic blood pressure (mm Hg)												
Median	172	160	156	128	170	165	140	165	150	148	133	160
Range (minimum-maximum)	110-230	121-280	120-220	107-150	128-230	120-244	140-150	140-220	116-200	140-160	120-176	101-210
IQR (Q1-Q3)	150-195	150-168	140-166	107-150	154-179	150-180	140-145	151-180	130-166	144-154	123-152	139-170

Abbreviations: IQR, interquartile range (expressed as quartile 1 [Q1] to quartile 3 [Q3]); MC, moderately controlled; N, number of animals; PC, poorly controlled; T1, T3, T6, and T12, 1, 3, 6, and 12 months after beginning of trilostane treatment, respectively; WC, well-controlled.

TABLE 5 Median alanine-aminotransferase and alkaline phosphatase concentrations at the different endpoints in all dogs, dogs that received receiving antihypertensive treatment (treated dogs, TD) and those not receiving antihypertensive treatment (untreated dogs, UTD). Superscript letters represent differences statistically significant (a: $P = .002$, b: $P = .00$, c: $P = .008$, d: $P = .004$)

		T6 (n = 45)			T12 (n = 37)		
	T0 (n = 51)	All (n = 45)	TD (n = 27)	UTD (n = 18)	All (n = 37)	TD (n = 23)	UTD (n = 14)
Alanine aminotransferase (U/L)							
Median	74[a,b]	48[a]	55	31	45[b]	45	36
Range (minimum-maximum)	15-670	10-162	17-162	10-144	10-201	16-157	10-201
IQR (Q1-Q3)	48-137	28-80	39-95	22-71	25-67	33-72	20-58
Alkaline phosphatase (U/L)							
Median	617[c,d]	464[c]	580	276	574[d]	604	544
Range (minimum-maximum)	36-6238	52-6914	52-4927	52-6914	58-6238	58-4278	83-6238
IQR (Q1-Q3)	156-1276	117-1133	97-1049	210-1242	157-992	88-1046	198-970

Abbreviations: IQR, interquartile range (expressed as quartile 1 [Q1] to quartile 3 [Q3]); N, number of animals; T0, diagnosis; T6 and T12, 6 and 12 months after beginning of trilostane treatment.

160 and 179 mm Hg). Hypertension was persistent in 5/9 dogs. Thus, antihypertensive treatment was prescribed in 5/15 (33.3%) NHT dogs at T0. Treatment was prescribed at T1 in 1/5 dogs (prehypertensive at T0), at T3 in 1/5 dogs (prehypertensive at T0), at T6 in 2/5 dogs (normotensive at T0), and at T12 in the remaining dog (normotensive at T0). Of these 5 dogs, 1 had mitral valve disease stage B1 before PDH diagnosis, which progressed to B2 at approximately the same time SH was observed. Another dog had leishmaniasis before inclusion in the study and developed IRIS stage 2 chronic kidney disease during the study period. The 3 remaining dogs did not have any concurrent conditions, before or during the study period.

Resolution of SH (SBP < 160 mm Hg) in dogs originally HT at T0 was achieved in 17/31 (54.8%) dogs at T6 (8/15 [53.3%] dogs moderately HT and 9/16 [56.25%] dogs severely HT). At T12, resolution of SH was achieved in 12/25 (48.0%) dogs originally HT (4/10 [40%] dogs moderately HT and 8/15 [53.3%] dogs severely HT).

Liver enzyme activity evaluated (ALT and ALKP) significantly decreased from T0 to T6 and T12, but not between T6 and T12. No significant differences were observed between dogs on antihypertensive treatment and those untreated at any time point. Complete data are presented in Table 5.

4 | DISCUSSION

The prevalence of SH at diagnosis was 70.6%, similar to previously reported results in dogs with HAC (31%-86%) and in humans (70%-85%) with CS.[4-6,8,9,11-14] The prevalence of severe SH was 39.2%, similar to recently reported results[13] but more common than observed by others,[8] probably because of differences in the cutoff values used to define severe SH.

Signalment, clinical signs, and physical examination findings were similar to those described in dogs with HAC.[1,2,37,38] None of these features, with the exception of age, were related to SH or with SBP results, which also is in agreement with the findings of others.[8,13] Contrary to previously reported results,[8,13] a correlation between age and SH was found. An effect of age on SBP occasionally has been observed in adults with CS,[33] but also in the general population.[39] Thus, it is uncertain, whether or not this relationship is independent of CS. The association between aging and SBP in dogs is unclear, and minor increases of 1 to 3 mm Hg have been found in some studies.[35,40] The design of our study did not allow us to independently assess the correlation between SBP and age in dogs with PDH, because a group of age-matched healthy controls would have been necessary for this purpose. Thus, it remains unclear whether this correlation is related to HAC itself or an aging effect on SBP in dogs. Concurrent diseases also were not related to SH.

The prevalence of SH significantly decreased during the study period, from 70.6% at T0 to 45.9% at T12, similar to what has been reported in humans after successful treatment.[4-7] Nevertheless, the prevalence of SH at the end of the study still was high. The prevalence of severe SH also significantly decreased from 31.4% at T0 to 10.8% at T12. Mean SBP showed a similar pattern, significantly decreasing from 170 to 152 mm Hg from T0 to T12. Changes in mean SBP during the study period were influenced by the risk of TOD at T0. Dogs severely HT at diagnosis experienced more pronounced decreases in mean SBP, whereas dogs normotensive at T0 tended to experience a slight increase in SBP. Previous studies in dogs with PDH treated with mitotane or trilostane have not reported significant differences in SBP or prevalence of SH before and after treatment.[8-10] A significant decrease in SBP has been described in well-controlled dogs treated with mitotane.[8] To interpret the discrepancies among these previous studies, 2 conditions should be considered. First, the higher proportion of severely HT dogs in our study (probably because of the different criteria used to classify SH) compared to those previously cited[8,9] could have led to a more pronounced decrease in SBP because these dogs tend to be treated earlier in the course of SH. Second, in both cited studies, it is not clearly addressed whether the dogs were treated with antihypertensive drugs, and if so, which medications were used,[8-10] and thus the results are difficult to

compare. In our opinion, the decrease in SBP and the prevalence of SH more likely might be related to antihypertensive treatment than to the cortisol-lowering effect of trilostane. The high prevalence of SH at the end of the study period also could indicate that the metabolic and vascular changes related to hypercortisolemia persisted despite medical treatment, as described in humans.[4,6,7] This possibility however was not investigated in our study and further research would be needed to evaluate it. The decrease in SH prevalence might have been due to HT dogs dying earlier in the course of their disease. However, because death at any time point was not related to blood pressure, this was not considered as a potential bias for our results.

Because the results of the ACTH-st correlate poorly with clinical signs in dogs with HAC treated with trilostane,[41-43] for the purpose of our study dogs were classified based on clinical signs reported by the owners. No correlation was observed between the prevalence of SH or mean SBP and control of the disease at any time point. In dogs with PDH treated with mitotane, a significant decrease in SBP is achieved only in well-controlled dogs, but the prevalence of SH in these dogs 3 months after treatment (approximately 40%)[8] is similar to that observed in our study for WC dogs. These findings are also similar to those reported in humans with CS, in whom even years after successful treatment SH still persists in 20% to 40% of patients.[7,15-19,22-27,31,44] Nevertheless, the number of WC dogs in our study significantly increased with time, thus decreasing the number of MC and PC dogs at T3, T6, and T12. Thus, it should be considered that the small number of MC and PC dogs at these endpoints may have led to type II errors.

Platelet count was significantly higher in HT dogs at diagnosis, as recently described[13] in dogs with HAC. This finding has been hypothesized to be a consequence of the vasoconstrictor effects of erythropoietin or thromboxane-A2.[13,45-47] However, this relationship was not observed at T12. In the original design of our study a CBC was not included at every visit. It would be interesting to evaluate the changes in platelet count during the course of the disease and its relationship to SBP.

Serum potassium concentrations were negatively correlated with SBP at T0 but not at the remaining time points. The relationship between lower serum potassium concentrations and SBP at diagnosis in dogs and humans with hypercortisolism has been suggested to be the result of an apparent mineralocorticoid excess, due to the nonselective binding of cortisol to MRs.[4-6,13,32,33,48,49] Aldosterone and cortisol, but not cortisone, have the same affinity for MRs.[48,50] The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD), which catalyzes the reaction from cortisol to cortisone, is abundantly expressed to promote aldosterone binding to the MR in mineralocorticoid target tissues.[48-50] An enhancing effect of trilostane on 11β-HSD has been proposed in dogs with HAC in vivo, as well as in sheep adrenal glands in vitro.[51,52] This accelerating effect on cortisol to cortisone conversion might explain why serum potassium concentrations and SBP were correlated at T0, before trilostane was administered, but not at the remaining time points. However, the influence of antihypertensive drugs, especially benazepril, also must be considered.

Basal serum cortisol concentrations were not correlated with SBP at T0, but a relationship was found at T1, T3, T6, and T12. One-hour post-ACTH serum cortisol concentrations were only correlated with SBP at T12. This lack of correlation between serum cortisol concentrations and SBP at diagnosis has been described previously in dogs and humans with CS.[4-8,11-13,15,19,24,33] The relationship between serum cortisol concentrations and SBP during trilostane treatment in dogs with HAC has not been evaluated previously. Currently, no objective method is available to evaluate the response to trilostane treatment in dogs with HAC, and serum cortisol concentrations correlate poorly with clinical signs.[41-43,53] However, the relationship between basal serum cortisol concentrations and SBP might indicate that the deleterious vascular effects of chronic hypercortisolism are more pronounced in dogs with higher basal serum cortisol concentrations, regardless of clinical control of the disease.

To achieve good control of blood pressure in humans with CS, normalization of hypercortisolism is essential, but antihypertensive drugs usually must be added to control SH.[4-7,24] Treatment with an ACEI or an ARB provides normalization of SBP in 50% of HT people with CS.[6,54,55] During the study period, 60% of the dogs required treatment with an ACEI (ie, benazepril), and of these dogs, 41.9% needed an additional drug (ie, amlodipine), similar to what is described in people.[6,54] In veterinary medicine, telmisartan has proven to be effective in managing SH in cats[56] and, in combination with amlodipine, in dogs with SH refractory to treatment with ACEIs.[57] In HT dogs with HAC, telmisartan has been shown to control SH in 40% of dogs, whereas benazepril has been effective in 80% of the cases (González, S; Clares, I; Alonso, D; García del Real, R; García, P; Pérez, MD "Telmisartan versus benazepril on the management of systemic hypertension in dogs with hyperadrenocorticism" Research communications of the 28th ECVIM-CA congress). Our results could have been different if telmisartan had been used. Further research evaluating the efficacy of ARBs in glucocorticoid-induced SH in dogs is necessary.

In the HT dogs at T0, resolution of SH was achieved in approximately 50% of them at T6 and T12. These findings are similar to findings in humans with CS after surgical treatment.[4-7,15-20] In people with pituitary-dependent CS (ie, Cushing's disease [CD]) treated medically, treatment with pasireotide, mifepristone, metyrapone or ketoconazole improved blood pressure in 20% to 70% of patients.[22-27] However, with some of these drugs, hypertension can worsen because of MR activation by 11-deoxycortisol, and treatment with a MR agonist is necessary.[26,27] Only cabergoline treatment results in complete resolution of SH in people with recurrence of CD after surgery, but this effect is thought to be related to the decrease in peripheral vascular resistance produced by cabergoline itself.[21] Antihypertensive treatment could not be decreased in any dog, but dogs previously treated with these drugs were not included, whereas in the majority of studies in people with CD, SH is treated before inclusion. In patients with CS, increased arterial stiffness is present at diagnosis and after treatment, and has been proposed as a possible mechanism of persistent SH in these patients, independent of the severity of SH at diagnosis.[16,17,28,29] Increased renal vascular resistance has been described in dogs with HAC, and in dogs with

concurrent HAC and diabetes mellitus.[58,59] Persistent increased arterial stiffness in dogs with HAC, secondary to glucocorticoid-induced vascular remodeling, might contribute to the inability to decrease antihypertensive treatment in this group of dogs.

Information regarding the changes in SBP in people or dogs with hypercortisolism and NHT at diagnosis is lacking. In our study, 60% of the NHT dogs at T0 had a SBP ≥ 160 mm Hg at some time point during the study period, and 33.3% of NHT dogs at diagnosis were persistently HT during follow-up, requiring treatment with benazepril. In 1 of these dogs, the occurrence of SH during the study period also might have been related to concurrent leishmaniasis that led to chronic kidney disease.[60] However, in the remaining dogs, we failed to identify an additional explanation for SH other than PDH. Increased arterial stiffness is described in NHT people with CS at diagnosis.[29] Nevertheless, to our knowledge, the role of these vascular changes in subsequent changes in SBP in patients NHT at diagnosis has not been evaluated.

Liver enzymes decreased significantly during the study, and at both T6 and T12, they were not different between dogs treated with benazepril and those untreated, similar to what was observed in a previous study of benazepril treatment in dogs with congestive heart failure.[61] Our results suggest that no increases in ALT and ALKP activity occur during benazepril treatment in dogs with PDH.

Our study had some limitations. The effects of trilostane on SBP cannot be fully evaluated because of the study design as dogs were treated with antihypertensive drugs when needed, and changes in SBP are more likely related to these agents. It would have been unethical however not to treat hypertension, especially in those dogs with higher risk of TOD. Also, no available device is completely validated for SBP measurement in conscious dogs[35] and using oscillometry could have led to different results. Another potential limitation is that currently no objective method is available to evaluate response to trilostane treatment, and thus results might have been different if response to treatment would have been evaluated by other means. In our study, dogs were treated with benazepril alone or in combination with amlodipine. Other drugs such as ARBs or aldosterone antagonists could have led to different results, and their efficacy to treat SH associated with hypercortisolism should be investigated further.

In conclusion, in dogs with PDH, antihypertensive drugs decrease SBP and the prevalence of SH during the first year of trilostane treatment. However, the prevalence of SH 1 year after treatment still is high (45.9%). Prevalence of SH and mean SBP are not correlated with clinical control of the disease, based on owner assessment. Resolution of SH was achieved in approximately 50% of the dogs initially HT, but some dogs required several antihypertensive drugs to manage SH. One third of dogs NHT at diagnosis developed SH during the first year of trilostane treatment and needed antihypertensive treatment. These findings suggest that, in dogs with PDH, SBP should be measured at every visit, regardless of clinical control of the disease.

ACKNOWLEDGMENT

Funding provided by Royal Canin in veterinary endocrinology at the Veterinary Teaching Complutense of Madrid.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no Approved by the hospital board of the Veterinary Teaching Hospital Complutense. All owners signed a consent at admission at the center allowing to use the data from their pets for research purposes.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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How to cite this article: García San José P, Arenas Bermejo C, Alonso-Miguel D, Clares Moral I, Cuesta-Alvaro P, Pérez Alenza MD. Changes in systolic blood pressure in dogs with pituitary dependent hyperadrenocorticism during the first year of trilostane treatment. *J Vet Intern Med*. 2021;35:130–141. https://doi.org/10.1111/jvim.15978