Contrast-enhanced ultrasonographic evaluation of adrenal glands in dogs with pituitary-dependent hyperadrenocorticism

Pascalline Pey, DVM, PhD; Sylvie Daminet, DVM, PhD; Pascale Marcel Yvonne Smets, PhD; Luc Duchateau, PhD; Pauline De Fornel-Thibaud, DVM; Dan Rosenberg, DVM, PhD; Jimmy C. H. Saunders, DVM, PhD

Objective—To assess vascular changes induced by hyperadrenocorticism of hyperplastic adrenal glands in dogs via contrast-enhanced ultrasonography.

Animals—12 dogs with pituitary-dependent hyperadrenocorticism (PDH) and 7 healthy control dogs ≥ 7 years old.

Procedures—Dogs were assigned to the PDH and control groups and to small-breed (n = 6), medium-breed (4), and large-breed (9) subgroups. Contrast-enhanced ultrasonography of both adrenal glands in each dog was performed with IV injections of contrast agent. Time-intensity curves for the adrenal cortex, adrenal medulla, and ipsilateral renal artery of both adrenal glands were generated. Perfusion variables (time to peak [TTP], upslope of wash-in phase, and downslope of washout phase) were calculated.

Results—Contrast-enhanced ultrasonography revealed no qualitative difference between PDH and control groups. Quantitatively, TTPs were longer in the adrenal cortex and adrenal medulla of the PDH group, compared with values for the control group, particularly in the adrenal cortex and adrenal medulla of the small-breed subgroup. Washout downslopes were lower for the renal artery, adrenal cortex, and adrenal medulla of the small-breed subgroup between the PDH and control groups. No other perfusion variables differed between groups.

Conclusions and Clinical Relevance—Contrast-enhanced ultrasonography of the adrenal glands in dogs with PDH revealed a delayed TTP in the adrenal cortex and adrenal medulla, compared with values for control dogs. Contrast-enhanced ultrasonography was able to detect vascular changes induced by hyperadrenocorticism. Further studies are needed to evaluate whether reference ranges for clinically normal dogs and dogs with PDH can be determined and applied in clinical settings. (*Am J Vet Res* 2013;74:417–425)

States of chronic ACTH excess (eg, PDH) are associated with hyperplasia and hypertrophy of the adrenocortical cells.[1,2] Equal hyperplasia of the compact cells of the zona reticulate and the clear cells of the zona fasciculate is evident during histologic examination; consequently, the width and weight of the adrenal cortex are increased.[1,2] An irregular corticomedullary junction and medullary compression by a thickened cortex have been reported.[1] Diffuse adrenocortical enlargement is the most commonly observed ultrasonographic manifestation of PDH.[3]

In addition, the typical arrangement of the zones can be obscured by micronodules of cortical tissue that compress the surrounding cortex and medulla. These micronodules can be focal or diffuse and of extracapsular or juxtamedullar origin.[2] Macronodular enlargement of one of the poles of the adrenal gland is routinely seen and can be secondary to micronodular hyperplasia of

Abbreviations

B-mode	Brightness mode
LDDST	Low-dose dexamethasone suppression test
MPB	Mean pixel brightness
PDH	Pituitary-dependent hyperadrenocorticism
ROI	Region of interest
TTP	Time to peak

Received April 10, 2012.
Accepted September 10, 2012.
From the Departments of Medical Imaging of Domestic Animals and Orthopaedics of Small Animals (Pey, Saunders), Small Animal Medicine and Clinical Biology (Daminet, Smets), and Physiology and Biometry (Duchateau), Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium; and the Unité de médecine interne, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France (De Fornel-Thibaud, Rosenberg). Dr. Pey's present address is Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Montreal, St Hyacinthe, QC J2S 7C6 Canada.
Presented in poster form at the European College of Veterinary Internal Medicine Companion Animal Congress, Seville, Spain, September 2011.
Address correspondence to Dr. Pey (pascaline_pey@hotmail.fr).

the adrenal gland.[4] Focal nodules should not be mistaken for adrenal gland neoplasms during ultrasonography. Several studies[4–7] have elicited the difficulties in distinguishing adrenal-dependent hyperadrenocorticism from PDH in situations of equivocal adrenal gland asymmetry. Therefore, there is a need for a new diagnostic test to investigate those asymmetric situations.

Contrast-enhanced ultrasonography can be used to evaluate adrenal gland and renal perfusion in dogs.[8,9] Microbubble contrast media remain entirely within the vasculature, mix uniformly with circulating blood, and possess the same intravascular rheologic characteristics as RBCs when injected IV.[10] Tracking the transit of a bolus of microbubbles has allowed the identification of vascular changes associated with physiologic or pathophysiologic processes in various organs.[10] Normal adrenal glands give rise to diffuse intense and centrifugal contrast enhancement because of the intense vascularization with a rich medullary capillary circulation.[9] Hyperplastic adrenal glands, similar to any other organs with hyperplastic lesions, may have early wash-in and normal washout phases.[10]

In addition, contrast-enhanced ultrasonography might be useful for the diagnosis of hyperadrenocorticism and for confirming pituitary dependence in ambiguous situations (eg, nodular enlargement of a pole of an adrenal gland or height of the caudal pole within anticipated limits despite clinical suspicion of PDH). Before clinicians and researchers can investigate nodular enlargement via contrast-enhanced ultrasonography, it is necessary to describe contrast-enhanced ultrasonography of diffuse adrenocortical enlargement in dogs with PDH. The purpose of the study reported here was to describe the enhancement pattern in adrenal glands of dogs with PDH that had diffuse adrenocortical enlargement and to compare perfusion variables of PDH dogs with those of control dogs of similar age and breeds.

Materials and Methods

Animals—Twelve client-owned dogs with untreated PDH were included in the study (PDH group). Seven client-owned healthy dogs ≥ 7 years old were used as the control group. The number of dogs in the study was determined by the duration of the recruitment period (30 months). Control and PDH dogs were recruited from owners who volunteered to allow the inclusion of their dogs and provided consent for use of their dogs. A power analysis was not performed because no information was available on the expected difference or on the variance of the measurements. The prospective case-control study was conducted at Ghent University in Merelbeke, Belgium, and was performed in accordance with the Animal Care Ethical Committee of Ghent University (EC2011/049).

Hyperadrenocorticism was diagnosed on the basis of history, clinical signs, results of physical examination, biochemical changes, and consistent results from one or more of the following screening tests: LDDST, urinary cortisol-to-creatinine concentration ratio in 2 consecutive morning urine samples, or ACTH stimulation test. Pituitary dependence was determined when there were at least 2 of the following: LDDST results indicative of PDH, urinary cortisol-to-creatinine concentration ratio suppressed ≥ 50% after oral administration of dexamethasone, increase in plasma ACTH concentration, ultrasonographic evidence of 2 equally enlarged adrenal glands, or detection of a pituitary mass via CT or MRI. A CBC, biochemical analysis, and urinalysis were performed on all dogs. The PDH dogs were allowed to acclimate to their surroundings, and arterial blood pressure then was measured noninvasively with a Doppler technique.[11] Exclusion criteria were the presence of concurrent systemic infectious, neoplastic, or endocrine diseases. Adrenal gland asymmetry and nodular enlargement of an adrenal gland detected during ultrasonography were also exclusion criteria, as was uncooperative behavior of a dog. Cardiac disease was an exclusion criterion, except for dogs with subclinical disease (International Small Animal Cardiac Health Council class Ia and Ib).[12]

To avoid potential confounding effects attributable to size-dependent or breed-dependent variations in adrenal gland perfusion, the number of healthy dogs was matched proportionally to the number of dogs with PDH for each of the following subgroups: small-breed dogs (< 12 kg), medium-breed dogs (12 to 20 kg), and large-breed dogs (> 20 to 40 kg). Four PDH dogs and 2 control dogs weighed < 12 kg, 2 PDH dogs and 2 control dogs weighed between 12 and 20 kg, and 6 PDH dogs and 3 control dogs weighed between 20 and 40 kg.

The minimum age of recruitment for control dogs was 7 years because PDH most commonly affects dogs > 7 years old.[13] Dogs from the control group were considered healthy on the basis of history and results of physical examination, a CBC, biochemical analysis, urinalysis, and arterial blood pressure measurement. Control dogs with 2 consecutive tests in which the urinary cortisol-to-creatinine concentration ratio was < 8 were included in the study.

Endocrine testing—Urine samples were collected via cystocentesis. Blood samples were collected from a jugular vein. An ACTH stimulation test was performed to determine the serum cortisol concentration before and 1 hour after IM injection of 1 mL of tetracosactide.[a] Serum cortisol concentrations were measured with a chemiluminescence method.[b] The LDDST, based on IV administration of dexamethasone[c] at a dose of 0.01 mg/kg, and the oral high-dose dexamethasone suppression test, based on oral administration of dexamethasone[d] at a dose of 0.1 mg/kg, were performed as described elsewhere.[14–18] Hyperadrenocorticism was confirmed by a marked increase (≥ 500 nmol/L) in serum cortisol concentration by 1 hour after ACTH injection, an inadequate decrease (≥ 40 nmol/L) in serum cortisol concentration by 8 hours after dexamethasone administration, or both. Endogenous ACTH concentrations were measured with an immunoluminometric assay.[19] Blood samples for ACTH determination were collected from a jugular vein into EDTA-coated tubes. Samples were immediately centrifuged at 500 × *g* for 8 minutes at 4°C, and the resulting plasma was transferred to plastic tubes and stored at –80°C until analyzed. Plasma ACTH concentrations were determined with an ACTH analyzer[e] validated for use in samples obtained from dogs.[20]

Pituitary dependence was determined when the endogenous ACTH concentration in plasma was ≥ 6 pg/mL.[19] Creatinine concentrations in urine were measured via a modified Jaffé method; cortisol concentrations in urine were measured with a chemiluminescence method.[f]

Diagnostic imaging—Pituitary dependence was supported in some dogs from the PDH group on the basis of results of CT or MRI of the pituitary gland. A pituitary gland height–to–brain area ratio > 0.31 mm^{-1} was used to identify an enlarged pituitary gland.[21,22]

Ultrasonographic images were obtained for both adrenal glands. Briefly, dogs were manually restrained in dorsal recumbency; dogs were not sedated for ultrasonography. Hair was clipped from the abdominal region. Coupling gel was applied, and B-mode ultrasonography[g] was performed with a 5-MHz linear transducer. Ultrasonographic images were obtained for all dogs via identical settings, except for overall gain, which was adjusted for each dog. Color Doppler ultrasonography was used to locate vessels adjacent to the adrenal glands, which were used as anatomic references. Echogenicity of the adrenal glands was assessed by comparison with the adjacent renal cortex or spleen. Echotexture (fine or granular), shape (peanut hull, boomerang, or other shape), and contour (smooth or rough) of the adrenal glands were recorded. Maximum length of the entire adrenal gland and maximum height of the cranial and caudal pole were measured with electronic calipers on longitudinal images of each adrenal gland in each dog. Measurements were obtained via a method described elsewhere.[23] The use of a cutoff value of 7.5 mm in large- and medium-breed dogs and 6.0 mm in small-breed dogs for the height of the caudal pole led to a strong suspicion of hyperadrenocorticism and was an inclusion criterion.[24,25,h] Adrenal asymmetry (an exclusion criterion) was based on a difference of > 3 mm in thickness of the caudal pole on longitudinal images between the 2 adrenal glands, and nodular enlargement of an adrenal gland (another exclusion criterion) was supported by a difference of > 3 mm between the 2 poles.

Contrast-enhanced ultrasonography of both adrenal glands was performed by the same examiner (PP), who used a technique described elsewhere.[9] A 20-gauge catheter was then placed into the left cephalic vein. The dogs were not sedated; they were manually restrained in dorsal recumbency. Contrast-enhanced ultrasonography was performed with a 5-MHz linear transducer and contrast-specific software.[i] This ultrasonographic examination involved the use of an annihilation method.[i] The mechanical index was 0.10, which corresponds to an acoustic pressure of 45 kPa (automatically set by the machine). In each dog, the overall gain and the time-gain compensation were adjusted such that there was no signal from the underlying parenchyma or other organs. The mean gain value was 61% (range, 56% to 63%). A single focal zone was placed in the deepest part of the image and was not changed during the injection.

The transducer was held in exactly the same position throughout the contrast ultrasonography. Images of the adrenal glands were obtained via the longitudinal axis. The phrenicoabdominal vessels, left renal artery, cranial mesenteric and celiac arteries (when possible), and aorta for the left adrenal gland and caudal vena cava and the right renal artery for the right adrenal gland were included in the images to serve as reference points.

Each dog received 3 injections of contrast agent. An aqueous suspension of phospholipid-stabilized lipid-shelled microbubbles filled with sulfur hexafluoride[j] was used as the contrast agent. The solution (5 mg/mL) was prepared < 2 hours before the start of the examination and was placed into a syringe for a maximum of 2 minutes before injection. The contrast agent was kept in a closed system before use. It was injected IV as a bolus (0.04 mL/kg), and the catheter was then flushed with 3 mL of saline (0.9% NaCl) solution. A 3-way stopcock was used to avoid any delay between injection of the contrast agent and saline solution. The timer was activated for each examination at the moment injection of the contrast agent began (time 0), and flow of the contrast agent into the adrenal glands was observed in real time.

The first injection of contrast agent was performed, and images of the left adrenal gland were obtained for 2 minutes. The ultrasonographic system was reset to maximal acoustic pressure, and the cranial aspect of the abdomen was scanned so that residual or trapped microbubbles would be destroyed. Absence of visual evidence of a microbubble signal within the abdominal portion of the aorta indicated the destruction of almost all circulating microbubbles. Images obtained after the first injection were not used for interpretation. Because all microbubbles from any previous injections may not be destroyed, images obtained after the first injection of contrast agent should not be compared with images obtained after any subsequent injections of contrast agent.[26] Therefore, a second injection of contrast agent was performed, and images of the left adrenal gland were obtained for 2 minutes. The time from the end of the first injection until beginning of the second injection was approximately 5 minutes. The procedure was repeated with a third injection of contrast agent, which was followed by evaluation of the right adrenal gland. A video of the ultrasonographic images obtained after each of the 3 injections was recorded digitally on a magnetic optic disk in the digital imaging and communications in medicine (ie, DICOM) format.

Subjective and objective assessments of images obtained after the second and third injections were performed. First, visual subjective evaluation was performed during and after the injections to ensure the sequence of events was clear. During this subjective evaluation, enhancement of the adrenal gland (cortex and medulla), renal artery, aorta, caudal vena cava (when visible), and phrenicoabdominal vessels was observed. Onset, duration, and intensity of the enhancement were recorded. The directions of the enhancement (ie, centripetal or centrifugal) and changes in relative brightness and homogeneity over time were also evaluated.

An objective quantitative computerized analysis was performed on images of the left adrenal gland obtained after the second injection of contrast agent and of the right adrenal gland obtained after the third injection of contrast agent. For both sequences, an ROI was manually drawn in the ipsilateral renal artery, adrenal cortex, and adrenal medulla. The area of the ROI was

Table 1—Mean ± SD measurements for both adrenal glands of 12 dogs with PDH and 7 healthy control dogs.

		Dogs with PDH		Control dogs	
Subgroup	**Variable**	**Left adrenal gland**	**Right adrenal gland**	**Left adrenal gland**	**Right adrenal gland**
Small breed (n = 6)	Cranial pole height (mm)	7.6 ± 1.6	7.5 ± 1.1	4.7 ± 0.1	4.7 ± 0.1
	Caudal pole height (mm)	8.0 ± 1.6	8.9 ± 1.9	5.1 ± 0.3	5.1 ± 0.3
	Length[a] (mm)	24.4 ± 3.2	24.7 ± 1.8	16.7 ± 3.6	16.7 ± 3.6
Medium breed (n = 4)	Cranial pole height (mm)	7.5 ± 0.5	9.4 ± 1.9	5.9 ± 1.4	7.1 ± 0.1
	Caudal pole height (mm)	8.1 ± 2.6	7.0 ± 2.4	6.4 ± 1.3	6.5 ± 0.2
	Length[a] (mm)	21.4 ± 6.9	23.0 ± 12.2	25.4 ± 2.9	24.1 ± 0.8
Large breed (n = 9)	Cranial pole height (mm)	8.7 ± 2.3	9.4 ± 3.3	6.6 ± 1.0	6.2 ± 1.7
	Caudal pole height (mm)	9.6 ± 2.6	8.4 ± 2.1	6.9 ± 0.7	6.2 ± 1.1
	Length[b] (mm)	32.6 ± 2.9	24.3 ± 5.1	25.5 ± 4.6	22.3 ± 3.4

Dogs with PDH comprised 4 small-breed dogs, 2 medium-breed dogs, and 6 large-breed dogs, whereas control dogs comprised 2 small-breed dogs, 2 medium-breed dogs, and 3 large-breed dogs. Body weight was < 12 kg, 12 to 20 kg, and > 20 to 40 kg for small-breed, medium-breed, and large-breed dogs, respectively.

[a,b]Within a variable, different superscript letters indicate significant ($P < 0.05$) differences between breed subgroups.

0.015 cm^2 and was similar in size and location for each region and each dog. Each image was displayed in an orange hue. The MPB was obtained via an image analysis system[k] and evaluated for each of the 3 ROIs (adrenal cortex, adrenal medulla, and ipsilateral renal artery). The MPB was measured every second from 0 to 40 seconds and at 60, 80, 100, and 120 seconds, and the values were entered into a spreadsheet file.[l] The background signal-intensity value (noise) corresponded to an MPB of 3. This was determined by placing an ROI (and measuring its color scale) in each region at time 0. The MPB at each time point was displayed in a time-intensity curve. Images displayed with software[k] were at a bit depth of 8 bits/pixel, which provided 256 potential values for each pixel.

The investigator was unaware of the endocrinologic status of each dog during the analysis. The TTP, wash-in upslope, and washout downslope were calculated for the adrenal cortex, adrenal medulla, and renal artery of both adrenal glands. The ratios of the MPB for the adrenal cortex to the MPB for the renal artery and the ratios of the MPB for the adrenal medulla to the MPB for the renal artery were calculated over time.

Statistical analysis—Normality of the data was tested via the Shapiro-Wilk test. Because the normal distribution assumption could not be rejected, a mixed model with group, side, and subgroup as categorical fixed effects and dog as a random effect was fitted to the data. All tests were based on a global value for significance of 5%; the value for significance for multiple comparisons was reduced via a Bonferroni adjustment technique. The response variables were TTP, wash-in upslope, and washout downslope. Statistical analysis was performed with a standard computer software program.[m]

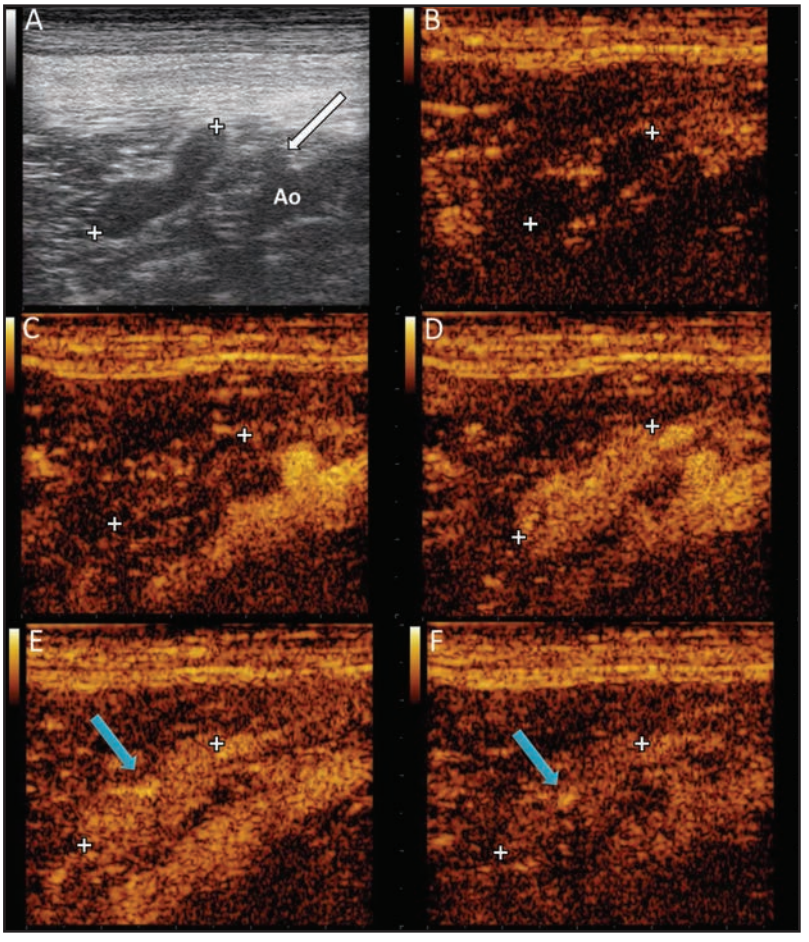

Figure 1—Sagittal B-mode (A) and contrast harmonic imaging (B–F) ultrasonographic images of the left adrenal gland of a dog with PDH. In each image, the adrenal gland is the structure between the plus signs; cranial is toward the left, and dorsal is toward the bottom. The transducer and machine were set for contrast harmonic imaging. A—Image acquired before injection of the contrast agent. The left renal artery (white arrow) is located caudoventral to the adrenal gland, and the aorta (Ao) is located dorsal to the adrenal gland. Notice the peanut hull shape of the adrenal gland and the phrenicoabdominal vessels in the hilum. B—Image acquired at the time of injection of the contrast agent. C—Image obtained 5 seconds after injection of the contrast agent. Notice the contrast-enhanced aorta, renal artery, and medullary parenchyma. D—Image obtained 15 seconds after injection of the contrast agent. Contrast enhancement is at its peak and extends centrifugally in the adrenal cortex. E—Image obtained 45 seconds after injection of the contrast agent. The contrast enhancement of the adrenal parenchyma is not evident, but there is persistent enhancement of the phrenicoabdominal vessels (blue arrow). F—Image obtained 120 seconds after injection of the contrast agent. A mild persistence of contrast agent is visible in the adrenal gland, phrenicoabdominal vessels (blue arrow), and aorta and is a result of microbubbles remaining in the systemic circulation.

All data were reported as mean ± SD unless otherwise indicated.

Results

Breeds represented in the PDH group were Labrador Retriever and Golden Retriever (n = 5), Jack Russell Terrier and Cairn Terrier (3), Beagle (2), Dachshund (1), and American Staffordshire Bull Terrier (1). Breeds represented in the control group were Foxhound (n = 2), Jack Russell Terrier (2), Beagle (2), and Labrador Retriever (1). The PDH group comprised 5 sexually intact males, 1 sexually intact female, 5 spayed females, and 1 neutered male. The control group comprised 1 sexually intact male, 3 spayed females, and 3 neutered males. Mean ± age of the dogs was 10 ± 2.2 years and 9 ± 1.7 years for the PDH and control groups, respectively. Mean body weight was 21.8 ± 13.2 kg and 17.3 ± 8.9 kg for the PDH and control groups, respectively.

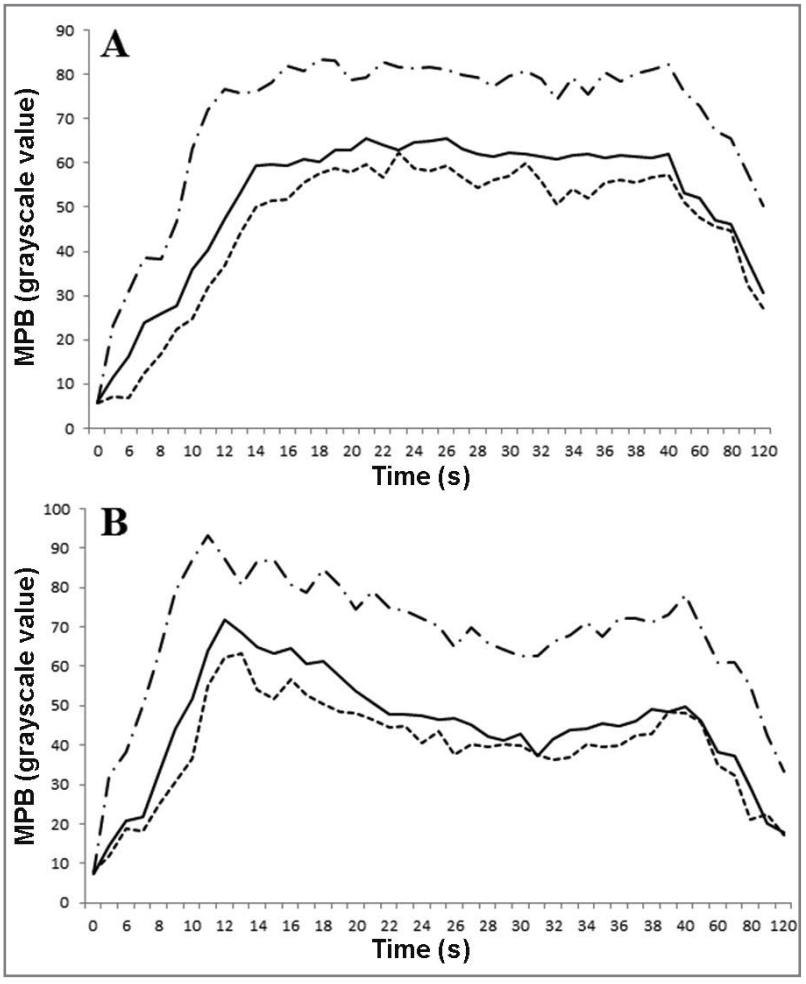

Figure 2—Values for MPB for the adrenal cortex (dashed line), adrenal medulla (solid line), and renal artery (dashed-and-dotted line) over time in 12 dogs with PDH (A) and 7 healthy control dogs (B). Data for the left and right adrenal glands were combined. The scale on the y-axis differs slightly between the panels. Notice the absence of the refilling phase in the dogs with PDH and the subsequent plateau. The upslope of the wash-in phase of the adrenal cortex and adrenal medulla is less steep and the peaks for the adrenal cortex and adrenal medulla are reached later in dogs with PDH than in the control dogs. Time 0 = Start of IV injection of contrast agent.

Evaluation of B-mode ultrasonographic images revealed that the adrenal glands from the PDH dogs had a peanut hull shape (n = 8), boomerang shape (2), or modified plump shape (14). Their contours were always smooth and the parenchyma homogeneously hypoechoic with that of the spleen, renal cortex, or surrounding adipose tissue. A corticomedullary distinction was recognized in only 2 dogs from the PDH group. Mean and SD of the measurements of both adrenal glands from dogs of the PDH and control groups were summarized (**Table 1**).

Contrast-enhanced ultrasonography revealed no subjective differences between the 2 groups, and dogs from both groups had a similar pattern of enhancement (**Figure 1**). The shape of time-intensity curves of adrenal and renal artery perfusion was different for the PDH and control groups (**Figure 2**). Mean and SD of the wash-in upslope, washout downslope, and TTP for the dogs of both groups were summarized (**Table 2**). For each variable and both groups, no significant differences were observed between the left and right adrenal glands. There were significant differences for the mean ± SD TTP of the adrenal cortex (6.6 ± 0.6 seconds vs 3.3 ± 0.8 seconds; P =

Table 2—Mean ± SD values for perfusion variables calculated from ROIs placed in the adrenal cortex, adrenal medulla, and renal artery of contrast-enhanced ultrasonographic images obtained from 12 dogs with PDH and 7 healthy control dogs.

Variable	ROI	Dogs with PDH	Control dogs	*P* value*
Wash-in upslope (grayscale value/s)	Adrenal cortex	10.0 ± 9.6	9.3 ± 3.6	0.514
	Adrenal medulla	8.0 ± 3.0	11.6 ± 4.5	0.117
	Renal artery	10.8 ± 2.6	14.3 ± 5.1	0.100
Washout downslope (grayscale value/s)	Adrenal cortex	–0.4 ± 0.1	–1.2 ± 0.9	0.555
	Adrenal medulla	–0.4 ± 0.1	–1.3 ± 1.0	0.964
	Renal artery	–0.4 ± 0.2	–2.1 ± 0.9	0.291
TTP (s)	Adrenal cortex	6.8 ± 2.9	3.4 ± 1.3	0.003
	Adrenal medulla	6.0 ± 2.7	4.3 ± 1.4	0.041
	Renal artery	5.7 ± 2.9	4.0 ± 2.0	0.525

*Values were considered significantly different at $P < 0.05$.

Table 3—Mean ± SD values for perfusion variables evaluated within the small-breed, medium-breed, and large-breed subgroups in Table 1.

Subgroup	Variable	ROI	PDH group	Control group	*P* value*
Small breed (n = 6)	Wash-in upslope (grayscale value/s)	Adrenal cortex	7.9 ± 1.6	10.5 ± 2.3	0.374
		Adrenal medulla	8.3 ± 1.9	13.1 ± 2.8	0.189
		Renal artery	9.7 ± 2.2	15.1 ± 3.1	0.169
	Washout downslope (grayscale value/s)	Adrenal cortex[a]	–0.4 ± 0.2	–1.7 ± 0.3	0.002
		Adrenal medulla[a]	–0.4 ± 0.2	–2.1 ± 0.3	0.001
		Renal artery[a]	–0.4 ± 0.2	–2.5 ± 0.3	< 0.001
	TTP (s)	Adrenal cortex	8.0 ± 0.9	4.0 ± 1.3	0.028
		Adrenal medulla[a]	7.7 ± 0.9	3.3 ± 1.3	0.016
		Renal artery	9.8 ± 1.3	10.2 ± 4.4	0.928
Medium breed (n = 4)	Wash-in upslope (grayscale value/s)	Adrenal cortex	13.8 ± 14.7	31.3 ± 32.1	0.389
		Adrenal medulla	9.6 ± 2.3	15.6 ± 2.3	0.121
		Renal artery	38.1 ± 19.7	84.6 ± 43.0	0.125
	Washout downslope (grayscale value/s)	Adrenal cortex[b]	–0.8 ± 0.4	–2.7 ± 0.9	0.165
		Adrenal medulla[b]	–0.5 ± 0.6	–0.4 ± 1.4	0.346
		Renal artery[b]	–1.4 ± 0.9	–4.6 ± 2.1	0.222
	TTP (s)	Adrenal cortex	3.0 ± 1.8	3.2 ± 1.7	0.116
		Adrenal medulla[a]	1.4 ± 6.6	7.9 ± 14.4	0.449
		Renal artery	1.6 ± 5.5	4.6 ± 12.1	0.521
Large breed (n = 9)	Wash-in upslope (grayscale value/s)	Adrenal cortex	33.1 ± 46.4	81.5 ± 81.5	0.332
		Adrenal medulla	10.3 ± 13.0	24.9 ± 22.8	0.228
		Renal artery	13.3 ± 2.5	10.3 ± 3.6	0.515
	Washout downslope (grayscale value/s)	Adrenal cortex[a,b]	–0.4 ± 0.07	–0.5 ± 0.1	0.224
		Adrenal medulla[b]	–0.4 ± 0.07	–0.5 ± 0.1	0.614
		Renal artery[a,b]	–0.4 ± 0.2	–0.9 ± 0.3	0.211
	TTP (s)	Adrenal cortex	4.2 ± 0.8	3.0 ± 1.2	0.419
		Adrenal medulla[b]	3.1 ± 0.5	2.5 ± 0.7	0.502
		Renal artery	2.4 ± 0.4	1.5 ± 0.5	0.224

See Tables 1 and 2 for key.

0.003) and adrenal medulla (6.0 ± 0.5 seconds vs 4.2 ± 0.6 seconds; $P = 0.04$) between the PDH and control groups, respectively. Despite differences in time-intensity curves, none of the other perfusion variables differed significantly between the 2 groups.

Mean and SD of the variables evaluated for the small-breed, medium-breed, and large-breed subgroups were summarized (**Table 3**). Mean ± SD TTP was significantly longer for the adrenal cortex (8.0 ± 0.9 seconds vs 4.0 ± 1.3 seconds; $P = 0.03$) and adrenal medulla (7.7 ± 0.9 seconds vs 3.3 ± 0.3 seconds; $P = 0.016$) of the PDH group, compared with values for the small-breed subgroup of the control dogs. The mean washout downslope was also significantly lower for the renal artery (–0.4 ± 0.2 vs –2.5 ± 0.3; $P < 0.001$), adrenal cortex (–0.4 ± 0.2 vs –1.7 ± 0.3; $P = 0.002$), and adrenal medulla (–0.5 ± 0.2 vs –2.1 ± 0.3; $P = 0.001$) for the PDH group, compared with results for the small-breed subgroup of the control dogs. No other significant differences were detected between the PDH and control groups for the medium-breed and large-breed subgroups. Changes over time for the ratios of the MPB for the adrenal cortex to the MPB for the renal artery and the MPB for the adrenal medulla to the MPB for the renal artery were plotted (**Figure 3**). The presence of a peak and a washout phase was more evident in the control group than in the PDH group, which had a plateau during the washout phase.

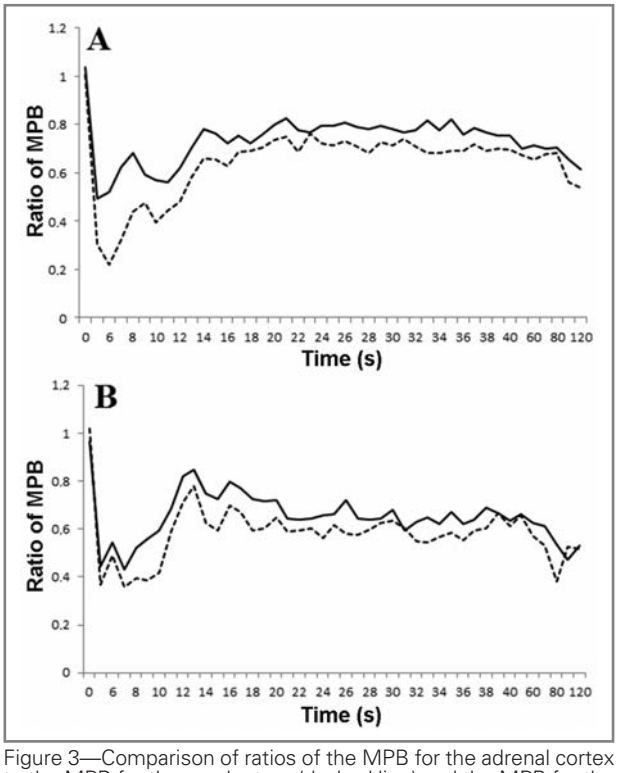

Figure 3—Comparison of ratios of the MPB for the adrenal cortex to the MPB for the renal artery (dashed line) and the MPB for the adrenal medulla to the MPB for the renal artery (solid line) for 12 dogs with PDH (A) and 7 healthy control dogs (B). Time 0 = Start of IV injection of contrast agent.

Discussion

Results of subjective and objective evaluations differed for contrast-enhanced ultrasonography in the present study. Although we did not detect differences between the 2 groups when viewing the video clip, time-intensity curves differed between the groups. Quantitative analysis should always be performed when contrast-enhanced ultrasonography is used to evaluate

perfusion of organs or lesions because it appears that visual observation might not be a reliable discriminator. We attempted to be as definitive as possible with subjective visual evaluation during and after the ultrasonographic evaluations until the sequence of events was clear, and we used a color scale for contrast-enhanced ultrasonography. However, because of the maximum sensitivity for luminance changes for the orange-yellow hue of the human visual system,[27] an objective (ie, quantitative) analysis was required.

Time-intensity curves for the PDH group had a delayed TTP, then a long plateau phase, and eventually an abrupt downslope representing the washout phase. The washout phase observed before the refilling time was not recognized in the PDH group, compared with results for the control group. In all veterinary studies[28–32] on contrast-enhanced ultrasonography of other organs, only qualitative analysis of lesions was performed. Visually, hyperplastic lesions (evident as nodular hyperplasia that can be observed in the liver or spleen) have a pattern of enhancement similar to that for normal hepatic or splenic parenchyma.[28–31,33] This was also observed for adrenal glands in the present study. However, it is difficult to explain the changes in the time-intensity curves. No information was found in the human literature because contrast-enhanced ultrasonography has not been reported in normal or hyperplastic human adrenal glands and the perfusion of adrenal glands in humans with PDH has not been described.

After IV injection of an ultrasonographic contrast agent, adrenal glands from dogs with PDH had a longer TTP, compared with the TTP in the clinically normal control dogs. There are 2 possibilities for those changes: effects of endogenous hypersecretion of glucocorticoids or morphological changes in hyperplastic adrenal glands. Endogenous hypersecretion of glucocorticoids induces vascular and hemodynamic changes; glucocorticoids may influence vascular tone, blood pressure, and electrolyte homeostasis through their mineralocorticoid activity and complex vasodilation and vasoconstriction activity.[33–36] Vascular and hemodynamic changes in patients with PDH are poorly understood. Glucocorticoids affect vascular tone, and patients with hyperadrenocorticism have increased sensitivity to angiotensin II and elevated plasma concentrations of the potent vasoconstrictor endothelin-1.[37,38] Glucocorticoids apparently downregulate the expression of the Na^+-Ca^{2+} exchanger in vascular smooth muscle cells, which causes an increase in the intracellular Ca^{2+} concentration and vasoconstriction.[39] This last factor may explain the reason that dogs with PDH in the study reported here had a delayed TTP. Mechanical compression of the medulla by hyperplastic adrenocortical tissue might also be a reasonable explanation for the delayed TTP in hyperplastic adrenal glands.

From a vascular point of view, extremely little information is available on the density and arrangement of blood vessels in hyperplastic adrenal glands. Cortical and medullary arteries can have microthrombi in their lumens.[2] Unfortunately, histologic analyses of the hyperplastic adrenal glands were not performed in the study reported here. An increase in the washout downslope was also noticed in the small-breed subgroup of dogs with PDH. However, we cannot explain the reason that small-breed dogs had an increase in washout downslope, compared with results for the medium-breed and large-breed dogs. The delayed TTP was especially evident in small-breed dogs. We do not have any reasonable explanation for that phenomenon, but the low number of dogs in the study may have contributed to the observed differences.

We did not detect differences in the time-intensity curves for the perfusion of the renal artery between the 2 groups. However, hyperadrenocorticism can cause hypertension in dogs and humans. Hypertension generally results from the interaction between several pathophysiologic mechanisms, including increased cardiac output, total peripheral resistance, and renovascular resistance.[40] In a recent study,[36] investigators found increased resistive and pulsatility indices (measured with ultrasonography) to be a sign of increased renal vascular resistance in dogs with PDH. In the study reported here, we did not detect differences in the shape of time-intensity curves or perfusion variables for the renal artery. Although we used older (≥ 7 years old) dogs for comparison, and dogs with renal disease (International Renal Interest Society stage ≥ 2)[41] were excluded from both groups, it is possible that dogs with mild renal disease were enrolled in both groups, which would explain why we did not detect differences in renal arterial perfusion.

In the present study, B-mode ultrasonography revealed that adrenal glands from dogs with PDH had a normal peanut hull shape or a modified plump shape. These findings are consistent with reported imaging characteristics for patients with PDH.[4,24,25,h] With regard to the size of the adrenal glands, only the length differed between subgroups in both the PDH and control groups. The adrenal glands of the small-breed subgroup of dogs with PDH were unexpectedly longer than were the adrenal glands of the medium-breed subgroup of dogs with PDH. However, given the small number of dogs in each subgroup, it is difficult to draw conclusions.

Height of the caudal pole of both adrenal glands in the small-breed and large-breed subgroups of control dogs was always below the published cutoff values for Yorkshire Terriers and Labrador Retrievers.[42] This observation supports the use of adapted cutoff values when examining dogs of different sizes and breeds.

Corticomedullary distinction was observed in only 2 dogs from the PDH group. However, it can also be seen in clinically normal dogs, and it does not constitute an imaging characteristic of PDH and is not correlated with histologic abnormalities.[43] Consequently, it was not used as an exclusion criterion.

Detection of vascular changes induced by PDH via contrast-enhanced ultrasonography of adrenal glands is feasible through analysis of time-intensity curves. Results of the present study should be confirmed in studies that include PDH patients with concurrent chronic disease, equivocal adrenal gland asymmetry, nodular enlargement, or adrenal gland size within reference values despite abnormal hormonal tests results. Contrast-enhanced ultrasonography could then potentially become a complementary technique to routine hormonal tests in selected

patients. Further studies will be needed to evaluate whether reference ranges for clinically normal dogs and dogs with PDH can be determined and applied in clinical settings.

a. Synacthen 0.25 mg/mL, tetracosactide hexa-acetate, Novartis Pharma, Vilvoorde, Belgium.
b. Architect i2000, Abbott Diagnostics, Lake Forest, Ill.
c. Rapidexon 2 mg/mL, dexamethasone phosphate, Eurovet NV/SA, Heusden-Zolder, Belgium.
d. Oradexon, dexamethasone, Organon Belgie, Brussels, Belgium.
e. Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, Calif.
f. ADVIA Centaur, CP immunoassay system, Siemens Healthcare, Brussels, Belgium.
g. CnTI Mylab 30, Esaote, Firenze, Italy.
h. Barthez PY, Nyland TG, Feldman EC. Ultrasonographic evaluation of the adrenal glands in normal dogs and dogs with pituitary-dependent hyperadrenocorticism (abstr). *J Vet Intern Med* 1994:8:160.
i. CnTI Megas Esatune, Esaote, Firenze, Italy.
j. Sonovue, Bracco Imaging, Milano, Italy.
k. ImageJ, 1.45m National Institutes of Health, Bethesda, Md. Available at: rsbweb.nih.gov/ij/docs/user-guide.pdf. Accessed Oct 17, 2012.
l. Windows 7, Microsoft Corp, Redmond, Wash.
m. SAS, version 9.2, SAS Institute Inc, Cary, NC

References

1. Capen CC, Martin SL, Koestner A. Neoplasms in the adenohypophysis in dogs. *Vet Pathol* 1967;4:301–325.
2. Kelly DF, Siegel ET, Berg P. The adrenal gland in dogs with hyperadrenocorticism. *Vet Pathol* 1971;8:385–400.
3. Schelling CG. Ultrasonography of the adrenal gland. *Probl Vet Med* 1991;3:604–617.
4. Grooters AM, Biller DS, Theisen SK, et al. Ultrasonographic characteristics of the adrenal glands in dogs with pituitary-dependent hyperadrenocorticism: comparison with normal dogs. *J Vet Intern Med* 1996;10:110–115.
5. Besso JG, Penninck DG, Gliatto JM. Retrospective ultrasonographic evaluation of adrenal lesions in 26 dogs. *Vet Radiol Ultrasound* 1997;38:448–455.
6. Hoerauf A, Reusch CE. Ultrasonographic characteristics of both adrenal glands in 15 dogs with functional adrenocortical tumors. *J Am Anim Hosp Assoc* 1999;51:193–199.
7. Benchekroun G, de Fornel-Thibaud P, Rodríguez Piñeiro MI, et al. Ultrasonography criteria for differentiating ACTH dependency from ACTH independency in 47 dogs with hyperadrenocorticism and equivocal adrenal asymmetry. *J Vet Intern Med* 2010;24:1077–1085.
8. Waller KR, O'Brien RT, Zagzebski JA. Quantitative contrast ultrasound analysis of renal perfusion in normal dogs. *Vet Radiol Ultrasound* 2007;48:373–377.
9. Pey P, Vignoli M, Haers H, et al. Contrast-enhanced ultrasonography of the normal canine adrenal gland. *Vet Radiol Ultrasound* 2011;52:560–567.
10. Haers H, Saunders JH. Review of clinical characteristics and applications of contrast-enhanced ultrasonography in dogs. *J Am Vet Med Assoc* 2009;234:460–470.
11. Brown S, Atkins C, Bagley R, et al. Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med* 2007;21:542–558.
12. Keene BW, Bonagura JD. Management of heart failure in dogs. In: Bonagura JD, Twedt DC, eds. *Kirk's current veterinary therapy*. 14th ed. St Louis: Saunders, 2009;771.
13. Feldman EC, Nelson RW. The adrenal gland. In: Feldman EC, Nelson RW, eds. *Canine and feline endocrinology and reproduction*. 3rd ed. Philadelphia: WB Saunders Co, 2004;251–484.
14. Meijer JC, de Bruijne JJ, Rijnberk A, et al. Biochemical characterization of pituitary-dependent hyperadrenocorticism in the dog. *J Endocrinol* 1978;77:111–118.
15. Meijer JC, Lubberink AA, Rijnberk A, et al. Adrenocortical function tests in dogs with hyperfunctioning adrenocortical tumours. *J Endocrinol* 1979;80:315–319.
16. Feldman EC. Comparison of ACTH response and dexamethasone suppression as screening tests in canine hyperadrenocorticism. *J Am Vet Med Assoc* 1983;182:506–510.
17. Feldman EC, Nelson RW, Feldman MS. Use of low- and high-dose dexamethasone tests for distinguishing pituitary-dependent from adrenal tumor hyperadrenocorticism in dogs. *J Am Vet Med Assoc* 1996;209:772–775.
18. Galac S, Kooistra HS, Teske E, et al. Urinary corticoid/creatinine ratios in the differentiation between pituitary-dependent hyperadrenocorticism and hyperadrenocorticism due to adrenocortical tumour in the dog. *Vet Q* 1997;19:17–20.
19. Rodríguez Piñeiro MI, Benchekroun G, de Fornel-Thibaud P, et al. Accuracy of an adrenocorticotropic hormone (ACTH) immunoluminometric assay for differentiating ACTH-dependent from ACTH-independent hyperadrenocorticism in dogs. *J Vet Intern Med* 2009;23:850–855.
20. Scott-Moncrieff JC, Koshko MA, Brown JA, et al. Validation of a chemiluminescent enzyme immunometric assay for plasma adrenocorticotropic hormone in the dog. *Vet Clin Pathol* 2003;32:180–187.
21. Kooistra HS, Voorhout G, Mol JA, et al. Correlation between impairment of glucocorticoid feedback and the size of the pituitary gland in dogs with pituitary-dependent hyperadrenocorticism. *J Endocrinol* 1997;152:387–394.
22. Auriemma E, Barthez PY, van der Vlugt-Meijer RH, et al. Computed tomography and low-field magnetic resonance imaging of the pituitary gland in dogs with pituitary-dependent hyperadrenocorticism: 11 cases (2001–2003). *J Am Vet Med Assoc* 2009;235:409–414.
23. Barberet V, Pey P, Duchateau L, et al. Intra- and interobserver variability of ultrasonographic measurements of the adrenal glands in healthy beagles. *Vet Radiol Ultrasound* 2010;51:656–660.
24. Barthez PY, Nyland TG, Feldman EC. Ultrasonographic evaluation of the adrenal glands in dogs. *J Am Vet Med Assoc* 1995;207:1180–1183.
25. Choi J, Kim H, Yoon J. Ultrasonographic adrenal gland measurements in clinically normal small breed dogs and comparison with pituitary-dependent hyperadrenocorticism. *J Vet Med Sci* 2011;73:985–989.
26. Delorme S, Krix M. Contrast-enhanced ultrasound for examining tumor biology. *Cancer Imaging* 2006;6:148–152.
27. Levkowitz H. Color scales for image data: design and evaluation. In: Levkowitz H, ed. *The international series in engineering and computer science*. Philadelphia: Springer Publishing, 1997;109–132.
28. Ohlerth S, Dennler M, Rüefli E, et al. Contrast harmonic imaging characterization of canine splenic lesions. *J Vet Intern Med* 2008;22:1095–1102.
29. Kanemoto H, Ohno K, Nakashima K, et al. Characterization of canine focal liver lesions with contrast-enhanced ultrasound using a novel contrast agent—Sonazoid. *Vet Radiol Ultrasound* 2009;50:188–194.
30. Nakamura K, Takagi S, Sasaki N, et al. Contrast-enhanced ultrasonography for characterization of canine focal liver lesions. *Vet Radiol Ultrasound* 2010;51:79–85.
31. Nakamura K, Sasaki N, Murakami M, et al. Contrast-enhanced ultrasonography for characterization of focal splenic lesions in dogs. *J Vet Intern Med* 2010;24:1290–1297.
32. Ortega TM, Feldman EC, Nelson RW, et al. Systemic arterial blood pressure and urine protein/creatinine ratio in dogs with hyperadrenocorticism. *J Am Vet Med Assoc* 1996;209:1724–1729.
33. O'Brien RT, Iani M, Matheson J, et al. Contrast harmonic ultrasound of spontaneous liver nodules in 32 dogs. *Vet Radiol Ultrasound* 2004;45:547–553.
34. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. *J Am Vet Med Assoc* 1998;212:369–373.
35. Goy-Thollot I, Pechereau D, Keroack S, et al. Investigation of the role of aldosterone in hypertension associated with spontaneous pituitary-dependent hyperadrenocorticism in dogs. *J Small Anim Pract* 2002;43:489–492.
36. Novellas R, de Gopegui RR, Espada Y. Determination of renal

vascular resistance in dogs with diabetes mellitus and hyperadrenocorticism. *Vet Rec* 2008;163:592–596.
37. Heaney AP, Hunter SJ, Sheridan B, et al. Increased pressor response to noradrenaline in pituitary dependent Cushing's syndrome. *Clin Endocrinol* 1999;51:293–299.
38. Martinez NI, Panciera DL, Abbott JA, et al. Evaluation of pressor sensitivity to norepinephrine infusion in dogs with iatrogenic hyperadrenocorticism. Pressor sensitivity in dogs with hyperadrenocorticism. *Res Vet Sci* 2005;78:25–31.
39. Smith L, Smith JB. Regulation of sodium-calcium exchanger by glucocorticoids and growth factors in vascular smooth muscle. *J Biol Chem* 1994;269:27527–27531.
40. Magiakou MA, Smyrnaki P, Chrousos GP. Hypertension in Cushing's syndrome. *Best Pract Res Clin Endocrinol Metab* 2006;20:467–482.
41. Polzin DJ. Chronic kidney disease. In: Ettinger SJ, Feldman EC, eds. *Veterinary internal medicine*. 7th ed. St Louis: Saunders, 2010;1992.
42. De Chalus T, Combes A, Bedu AS, et al. Ultrasonographic adrenal gland measurements in healthy Yorkshire Terriers and Labrador Retrievers. *Anat Histol Embryol* 2013;42:57–64.
43. Grooters AM, Biller DS, Merryman J. Ultrasonographic parameters of normal canine adrenal glands: comparison to necropsy findings. *Vet Radiol Ultrasound* 1995;36:126–130.