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

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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)
the adrenal gland. Focal nodules should not be mis-
taken for adrenal gland neoplasms during ultrasono-
graphy. Several studies have elicited the difficulties in
distinguishing adrenal-dependent hyperadrenocortici-
cism from PDH in situations of equivocal adrenal gland
asymmetry. Therefore, there is a need for a new diag-
nostic test to investigate those asymmetric situations.

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

In addition, contrast-enhanced ultrasonography might be useful for the diagnosis of hyperadrenocortici-
tism 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 ultraso-
nography, 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 ad-
renal glands of dogs with PDH that had diffuse adre-
nocortical enlargement and to compare perfusion vari-
ables of PDH dogs with those of control dogs of similar
age and breeds.

**Materials and Methods**

**Animals**: Twelve client-owned dogs with untreat-
ed 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 informa-
tion was available on the expected difference or on the
variance of the measurements. The prospective case-
control study was conducted at Ghent University in
Merebeke, 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 examina-
tion, 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 stimula-
tion test. Pituitary dependence was determined when
there were at least 2 of the following: LDDST results
indicative of PDH, urinary cortisol-to-creatinine concen-
tration ratio suppressed ≥ 50% after oral adminis-
tration 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 uri-
nalysis were performed on all dogs. The PDH dogs were
allowed to acclimate to their surroundings, and arte-
trial blood pressure then was measured noninvasively
with a Doppler technique. Exclusion criteria were the
presence of concurrent systemic infectious, neoplastic,
or endocrine diseases. Adrenal gland asymmetry and
nodular enlargement of an adrenal gland detected dur-
ing ultrasonography were also exclusion criteria, as was
uncooperative behavior of a dog. Cardiac disease was
an exclusion criterion, except for dogs with subclini-
cal disease (International Small Animal Cardiac Health
Council class la and Ib).

To avoid potential confounding effects attribut-
able 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 be-
tween 20 and 40 kg.

The minimum age of recruitment for control dogs
was 7 years because PDH most commonly affects dogs
> 7 years old. Dogs from the control group were con-
sidered healthy on the basis of history and results of
physical examination, a CBC, biochemical analysis, uri-
nalysis, and arterial blood pressure measurement. Con-
trol 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.
Serum cortisol concentrations were measured with a
chemiluminescence method. The LDDST, based on IV
administration of dexamethasone at a dose of 0.01 mg/
kg, and the oral high-dose dexamethasone suppression
test, based on oral administration of dexamethasone
at a dose of 0.1 mg/kg, were performed as described
elsewhere. Hyperadrenocorticism was confirmed by a
marked increase (≥ 500 nmol/L) in serum cortisol
concentration by 1 hour after ACTH injection, an inad-
equate decrease (≥ 40 nmol/L) in serum cortisol
concentration by 8 hours after dexamethasone adminis-
tration, or both. Endogenous ACTH concentrations
were measured with an immunoluminometric assay.
Blood samples for ACTH determination were collected
from a jugular vein into EDTA-coated tubes. Samples were
immediately centrifuged at 500 X 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 ana-
lyzer validated for use in samples obtained from dogs.
Pituitary dependence was determined when the endogenous ACTH concentration in plasma was ≥ 6 pg/mL.\textsuperscript{19} Creatinine concentrations in urine were measured via a modified Jaffé method; cortisol concentrations in urine were measured with a chemiluminescence method.\textsuperscript{1}

**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\textsuperscript{-3} was used to identify an enlarged pituitary gland.\textsuperscript{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\textsuperscript{6} 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, bootmerang, 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.\textsuperscript{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.\textsuperscript{24,25,26}

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.\textsuperscript{4} 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.\textsuperscript{8} This ultrasonographic examination involved the use of an annihilation method.\textsuperscript{1} 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\textsuperscript{1} 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.\textsuperscript{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.\textsuperscript{27}

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, centrifetal 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.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Variable</th>
<th>Dogs with PDH</th>
<th>Control dogs</th>
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</thead>
<tbody>
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<td></td>
<td>Left adrenal gland</td>
<td>Right adrenal gland</td>
<td>Left adrenal gland</td>
</tr>
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<td>Small breed</td>
<td>Cranial pole height (mm)</td>
<td>7.6 ± 1.6</td>
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<td>(&lt;6)</td>
<td>Caudal pole height (mm)</td>
<td>8.0 ± 1.6</td>
<td>8.9 ± 1.9</td>
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<td>(n = 4)</td>
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<td>7.0 ± 2.4</td>
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<tr>
<td>Large breed</td>
<td>Cranial pole height (mm)</td>
<td>8.7 ± 2.3</td>
<td>9.4 ± 3.3</td>
</tr>
<tr>
<td>(n = 9)</td>
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<td>9.6 ± 2.6</td>
<td>8.4 ± 2.1</td>
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<tr>
<td></td>
<td>Length (mm)</td>
<td>21.4 ± 6.9</td>
<td>23.0 ± 12.2</td>
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<td></td>
<td></td>
<td>32.6 ± 2.9</td>
<td>24.3 ± 5.1</td>
</tr>
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</table>

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.

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

0.015 cm² 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 imaging analysis system and evaluated for hue. 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. 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 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.
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.

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>
<thead>
<tr>
<th>Variable</th>
<th>ROI</th>
<th>Dogs with PDH</th>
<th>Control dogs</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash-in upslope</td>
<td>Adrenal cortex</td>
<td>10.0 ± 9.6</td>
<td>9.3 ± 2.6</td>
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<td>Adrenal medulla</td>
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<td>Washout downslope</td>
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*Values were considered significantly different at P < 0.05.
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

<table>
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<tr>
<th>Subgroup</th>
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<td>Adrenal medulla b 8.3 ± 1.9</td>
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<td>Renal artery c 9.7 ± 2.2</td>
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<td>Renal artery 2.4 ± 0.4</td>
<td>1.5 ± 0.5 0.224</td>
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See Tables 1 and 2 for key.
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, 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 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. 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. 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. Glucocorticoids apparently downregulate the expression of the Na⁺-Ca²⁺ exchanger in vascular smooth muscle cells, which causes an increase in the intracellular Ca²⁺ concentration and vasoconstriction. 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 micronicthrombi in their lumens. 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. In a recent study, 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) 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. 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 value for Yorkshire Terriers and Labrador Retrievers. 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. 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.

References