

# Effects of a mock ultrasonographic procedure on cortisol concentrations during low-dose dexamethasone suppression testing in clinically normal adult dogs

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**Objective**—To determine whether the stress of an ultrasonographic procedure would interfere with the suppressive effect of dexamethasone during a low-dose dexamethasone suppression test (LDDST) in healthy dogs.

**Animals**—6 clinically normal adult dogs.

**Procedure**—In phase 1, an LDDST was performed 5 times at weekly intervals in each dog. Serum samples were obtained 0, 2, 4, 6, and 8 hours after dexamethasone injection. A mock 20-minute abdominal ultrasonographic examination was performed on all dogs at each time point during the LDDST on weeks 2 through 5. In phase 2, serum cortisol concentrations were measured before and immediately after a 20-minute mock abdominal ultrasonographic examination, as described for phase 1.

**Results**—We did not detect significant differences after dexamethasone injection when comparing median cortisol concentrations for weeks 2 to 5 (mock ultrasonographic procedure) with median concentration for week 1 (no mock ultrasonographic procedure). For 5 of the 6 dogs, cortisol concentrations after dexamethasone injection decreased to < 35.9 nmol/L after each mock ultrasonographic procedure and remained low for the duration of the LDDST. In phase 2, all dogs had significant increases in cortisol concentrations immediately after the mock ultrasonographic procedure.

**Conclusions and Clinical Relevance**—A 20-minute mock abdominal ultrasonographic examination performed during LDDST did not alter results of the LDDST in most dogs. Cortisol concentrations measured immediately after a mock ultrasonographic examination were significantly increased. Ultrasonographic procedures should be performed a minimum of 2 hours before collection of samples that will be used to measure cortisol concentrations. (*Am J Vet Res* 2004;65:267–270)

Naturally developing hyperadrenocorticism (HAC) is a common endocrine disorder in dogs.<sup>1</sup> It can be challenging to diagnose. Diagnosis is made on the basis of information gathered from multiple sources, includ-

ing results of physical examination, a CBC count, serum biochemical analyses, urinalysis, an ACTH stimulation test, a low-dose dexamethasone suppression test (LDDST), and abdominal ultrasonography.<sup>2</sup> In most cases, more than 1 dynamic screening test is needed to establish the diagnosis of HAC.<sup>2,3</sup>

Both the LDDST and ACTH stimulation test are used as screening tests for the diagnosis of naturally developing HAC. The ACTH stimulation test is of 1 or 2 hours' duration and differentiates iatrogenic HAC from naturally developing HAC; it is also used to monitor the patient's response to administration of mitotane (o,p'DDD).<sup>1</sup> However, the ACTH stimulation test can yield false-negative results in dogs with HAC because it is less sensitive than the LDDST.<sup>2,4</sup> It has been reported that up to 15% to 20% of dogs with HAC will have a normal response to ACTH administration.<sup>1,4</sup> In addition, the ACTH stimulation test cannot be used to differentiate between pituitary-dependent HAC and cortisol-secreting adrenal tumors.<sup>1,4</sup> Because of the expense and intermittent unavailability of cosyntropin<sup>3</sup> as well as its lower sensitivity, the ACTH stimulation test may not be the ideal test for initially screening patients suspected of having HAC.

The LDDST is a test of 8 hours' duration with sensitivity of 100%<sup>2</sup> and a negative-predictive value of 100%<sup>5</sup> when used in dogs with clinical signs consistent with HAC. In addition, the LDDST is diagnostic for pituitary-dependent HAC when suppression is detected by 4 hours after injection and is followed by a return to values within the reference range.<sup>1,6</sup> However, the LDDST does not differentiate between iatrogenic HAC and naturally developing HAC.<sup>1</sup> The LDDST uses a readily available and inexpensive compound, is easy to perform and interpret, and is extremely sensitive in dogs suspected of having HAC.

Abdominal ultrasonography is often recommended as an additional screening test to aid in the diagnosis of naturally developing HAC.<sup>1,7</sup> It would be convenient to perform additional diagnostic testing, such as abdominal ultrasonography, during the 8-hour period required for the LDDST. Information gained from abdominal ultrasonographic procedures would aid clinicians in differentiating pituitary-dependent from adrenal tumor-dependent conditions. It has been suggested that this should be avoided because the stress of abdominal ultrasonography may interfere with the suppressive effect of dexamethasone<sup>8</sup>; however, we are not aware of any studies examining this effect. Therefore, the purpose of the study reported here was to determine whether the stress of an ultrasonographic

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procedure would interfere with the suppressive effect of dexamethasone in clinically normal dogs and whether there would be an optimal time to conduct an ultrasonographic examination in relation to an LDDST.

## Materials and Methods

**Animals**—Six healthy adult client-owned dogs were enrolled in the study; all dogs were used with informed consent of their owners. Dogs included 2 Dachshunds, 1 German Shepherd Dog, 1 Rottweiler, and 2 mixed-breed dogs (4 spayed females and 2 castrated males). Dogs ranged from 1.67 to 4.75 years of age (median, 3 years), and body weight ranged from 5.5 to 55.7 kg.

All dogs were deemed healthy on the basis of medical history provided by the owners and results of physical examination. Baseline values for a CBC count, serum biochemical analyses (including electrolytes), and urinalysis were determined before dogs were enrolled in the study to rule out concurrent underlying disease. Furthermore, these dogs did not have a history of oral or injectable administration of glucocorticoids. Administration of topical formulations of glucocorticoids was ceased a minimum of 3 weeks before enrollment in the study. Compounds for heartworm prevention and flea control were the only medications permitted during the study. The protocol was reviewed and approved by an institutional animal care and use committee.

**Study protocol**—The study was conducted in 2 phases. All dogs included in the study had cortisol concentrations that decreased to < 35.9 nmol/L at 4 and 8 hours after injection of dexamethasone during an initial LDDST (week 1 of phase 1).

During phase 1, an LDDST was performed 5 times in each dog at weekly intervals. A blood sample was collected from a jugular vein at time 0, after which a low dose of dexamethasone (0.01 mg/kg) was administered via a cephalic vein.<sup>1</sup> Additional blood samples were collected 2, 4, 6, and 8 hours after dexamethasone injection for each LDDST during weeks 1 through 5. For weeks 2 through 5, a mock abdominal ultrasonographic examination was performed on all dogs for each time point during the LDDST. The mock ultrasonographic procedure was not performed during week 1 because those results provided baseline data. To simulate abdominal ultrasonography, each dog was positioned in dorsal recumbency and the ventral portion of the abdomen was clipped. A mock ultrasonographic procedure that lasted approximately 20 minutes was performed by use of an ultrasound probe and lubricating gel in a darkened room. Dogs were randomly assigned to have the ultrasonographic procedure performed

approximately 20 minutes before collection of the blood sample 2, 4, 6, or 8 hours after dexamethasone injection; a blood sample was subsequently obtained within 5 minutes after completion of the ultrasonographic procedure. Dogs were not walked outdoors or fed during the 8-hour LDDST in an attempt to minimize additional stress during the test period.

Phase 2 was conducted beginning 1 to 4 months after completion of phase 1. Dexamethasone was not administered to the dogs during phase 2. Blood samples for measurement of cortisol concentrations were obtained from the jugular vein of each dog before and immediately after a 20-minute mock abdominal ultrasonographic procedure. The ultrasonographic procedure was conducted as described in phase 1.

**Cortisol assay**—Serum was harvested from all blood samples. To avoid interassay variation, serum samples were stored at -70°C until assayed for cortisol in batches by use of radioimmunoassay procedures<sup>b</sup> that have been validated for use in samples obtained from dogs.<sup>8</sup> Limit of detection of the assay was 5.5 nmol/L. All tests were performed in the clinical endocrinology laboratory at the University of Tennessee College of Veterinary Medicine.

**Statistical analysis**—Data were analyzed by use of a statistical software package.<sup>c</sup> Descriptive statistics were evaluated for all variables. A repeated-measures ANOVA was used to compare the median cortisol concentrations 0, 2, 4, 6, and 8 hours after injection of dexamethasone. When data were not normally distributed, the Friedman repeated-measures ANOVA on ranks was performed. When significance was detected, the Tukey test was used to evaluate all pairwise comparisons. Student paired *t* tests were used to compare cortisol concentrations before and after the mock ultrasonographic procedure. Significance for all tests was set at *P* < 0.05.

## Results

During phase 1, median cortisol concentration at time 0 was significantly greater than concentrations at all time points after dexamethasone administration (Table 1). We did not detect significant differences within each time point after dexamethasone injection when comparing median cortisol concentrations for weeks 2 through 5 (mock ultrasonographic procedure) with the concentration for week 1 (no mock ultrasonographic procedure). Median cortisol concentrations 2, 4, 6, and 8 hours after dexamethasone injection differed significantly, with the concentration 2

Table 1—Comparison of cortisol concentrations for 6 clinically normal dogs during a low-dose dexamethasone suppression test that were subjected to a mock abdominal ultrasonographic procedure during the test

Time of ultrasonographic procedure*	Time after dexamethasone injection (h)				
	0	2	4	6	8
No ultrasonography	46.9 <sup>a</sup> (26.5–60.7)	14.9 (14.3–16.8)	11.6 <sup>b</sup> (10.5–13.2)	11.9 <sup>b</sup> (10.5–12.7)	10.5 <sup>b</sup> (9.4–11.3)
2 hours	50.5 <sup>a</sup> (31.5–67.9)	15.7 (13.2–17.1)	11.6 <sup>b</sup> (10.8–13.0)	11.9 (9.9–12.4)	11.0 <sup>b</sup> (10.2–12.1)
4 hours	50.8 <sup>a</sup> (41.4–56.3)	16.0 (14.3–18.8)	12.1 (11.6–16.6)	11.3 <sup>a</sup> (9.9–12.1)	11.3 <sup>b</sup> (10.8–11.3)
6 hours	74.5 <sup>a</sup> (33.1–126.1)	17.1 (14.9–18.5)	11.9 (11.0–12.7)	12.4 (11.0–18.5)	12.1 <sup>b</sup> (10.5–12.4)
8 hours	43.6 <sup>a</sup> (34.5–53.5)	16.0 (14.6–17.1)	12.1 <sup>b</sup> (11.9–12.7)	11.6 <sup>b</sup> (11.0–12.7)	11.6 (11.3–18.8)

Values reported are median (25th to 75th percentiles) nanomoles per liter.

\*Dogs were positioned in dorsal recumbency, the ventral portion of the abdomen was clipped, and a mock abdominal ultrasonographic examination that lasted approximately 20 minutes was performed by use of an ultrasound probe in a darkened room. Ultrasonographic examinations were performed immediately before sample collection at the indicated time point.

<sup>a</sup>Within a row, concentration is significantly (*P* < 0.05) greater than the concentrations for samples obtained at 2, 4, 6, and 8 hours. <sup>b</sup>Within a row, concentration is significantly (*P* < 0.05) less than the concentration for the sample obtained at 2 hours.

hours after dexamethasone injection consistently the highest, regardless of the time of the ultrasonographic procedure.

When results were assessed on the basis of each dog, cortisol concentrations after dexamethasone injection remained decreased in 5 of the 6 dogs for the duration of the LDDST. More specifically, all cortisol concentrations measured at time points after dexamethasone injection for these dogs remained decreased following each mock ultrasonographic procedure. During weeks 2 and 3 of phase 1 (ie, first and second mock ultrasonographic procedures), 1 dog had cortisol concentrations that exceeded 35.9 nmol/L at 6 hours (week 2) and 4 hours (week 3) after dexamethasone injection (40.0 and 45.2 nmol/L, respectively). The mock ultrasonographic procedure had been performed immediately before both of those samples were obtained. However, by the subsequent time point, the cortisol concentrations for that dog had substantially decreased to 13.0 and 12.7 nmol/L, respectively; thus, the concentrations were well within the reference range.

During phase 2 of the study, all dogs had significant increases in cortisol concentrations immediately after the mock ultrasonographic procedure. Mean  $\pm$  SD cortisol concentration before the mock ultrasonographic procedure was  $38.9 \pm 14.9$  nmol/L, whereas mean concentration after the mock ultrasonographic procedure was  $88.8 \pm 49.1$  nmol/L.

## Discussion

The LDDST is a screening test commonly used to aid in the diagnosis of HAC. Recommended time points for collection of samples for the LDDST are 0, 4, and 8 hours after dexamethasone injection.<sup>1,3</sup> The importance of the sample at 8 hours (referred to as a screening test) is to determine whether a dog has HAC.<sup>1</sup> Cortisol concentrations in a clinically normal dog should remain decreased to  $< 35.9$  nmol/L for the entire 8-hour period. The sample obtained at 4 hours (referred to as a discrimination test) helps differentiate adrenal gland tumors from pituitary-dependent HAC.<sup>1</sup> A dog with pituitary-dependent HAC should have cortisol concentrations that are decreased to  $< 35.9$  nmol/L (or a decrease of  $\geq 50\%$  of the baseline value at 4 hours) but that then increase to  $> 35.9$  nmol/L by 8 hours, whereas a dog with an adrenal gland tumor should not have decreased concentrations at the 4-hour time point.<sup>6</sup> However, a small percentage of dogs with pituitary-dependent HAC will not have suppressed cortisol concentrations at the 4-hour time point.<sup>1</sup>

Analysis of results of the study reported here revealed that stress associated with a mock ultrasonographic procedure did not adversely affect cortisol concentrations after dexamethasone injection in a small population of clinically normal dogs. In only 1 dog did mock ultrasonography increase cortisol concentrations immediately after the ultrasonographic procedure (2 separate samples obtained in weeks 2 and 3). These induced changes could have resulted in an inaccurate diagnosis of HAC, especially if the ultrasonographic procedure was performed prior to collection of the

sample at the 8-hour time point. In this dog, the cortisol concentrations returned to a decreased concentration within 2 hours after the mock ultrasonographic procedure, suggesting that timing of procedures after dexamethasone injection is critical. On subsequent test weeks (4 and 5), the cortisol concentrations in this dog were adequately suppressed at all time points independent of the mock ultrasonographic procedure. It is possible that this dog adapted to the mock ultrasonographic procedure during the 4-week experimental period. This is unlikely because this dog had the highest cortisol concentration (142.9 nmol/L) after the mock ultrasonographic procedure during phase 2 of the study. In addition, it is possible that the weekly administration of a potent glucocorticoid may have had a suppressive effect on the subsequent testing of the hypothalamic-pituitary-adrenal axis, resulting in values that were decreased to within the reference range. However, we do not believe this was a factor based on results of other studies<sup>3,6,9</sup> that used similar washout periods between subsequent tests. In addition, the plasma half-life of dexamethasone is 1.6 to 5 hours,<sup>10</sup> and the dosage used in the study reported here was equivalent to a physiologic dose of prednisone. Also, after a large dose of dexamethasone (0.1 mg/kg) used for high-dose dexamethasone suppression tests, adrenocortical suppression lasts for 32 hours.<sup>11</sup> Because the dose we used was a tenth of that high dose, and the 32-hour time frame was substantially less than the 1-week washout period used in our study, we believe this was an appropriate protocol and were not concerned that there would be a carryover effect. We could not find references to support a carryover or period effect for dexamethasone when administered at a rate of 0.01 mg/kg.

When the mock ultrasound procedure was performed without preceding administration of dexamethasone (ie, phase 2), all dogs had significant increases in cortisol concentrations immediately after the ultrasonographic procedure. This is consistent with results of other studies<sup>12-14</sup> in which investigators evaluated serum cortisol concentrations before and after stressful events, such as intradermal allergy testing, routine skin scraping, collection of skin biopsy specimens, minor surgery, and anesthesia. Thus, routine procedures may affect cortisol concentrations in dogs equally.<sup>12-14</sup>

Other studies<sup>2,15</sup> have revealed that the hypothalamic-pituitary-adrenal axis does not respond as expected in dogs with chronic illness. Such patients have a blunted response to the suppressive effects of dexamethasone during the LDDST and an exaggerated response to ACTH.<sup>15</sup> Our study substantiates that clinically normal dogs do not respond in this manner because, in all likelihood, they had an acute ACTH surge with acute stress, such as that associated with an ultrasonographic procedure. This phenomenon has been documented in another study<sup>16</sup> and involves various factors, such as availability and metabolism of cortisol. Thus, dogs with chronic severe nonadrenal illness should not be tested for HAC until after they have recovered.

Analysis of results from the study reported here and other studies<sup>12-14</sup> suggests that minor medical pro-

cedures performed during an LDDST do not alter the results in most dogs. It may rarely cause a temporary increase in cortisol secretion in response to stress (as seen in 1 dog in our study); however the suppressive effect of dexamethasone then quickly caused a decrease in cortisol concentrations to within the reference range. Because of the small number of dogs used in this study, sweeping recommendations should not be made. Collecting this information from a larger population of clinically normal dogs as well as from dogs with HAC would strengthen the validity of the data reported here. Nonetheless, on the basis of these results, it is feasible to consider performing ultrasonographic procedures 2 or more hours before acquisition of samples that will be used to measure cortisol concentrations (hours 1 and 2 or 5 and 6 after dexamethasone administration). By keeping stressful medical procedures temporally close to time of dexamethasone administration (ie, prior to hours 1 and 2) or close to the 4-hour postdexamethasone sample (ie, prior to hours 5 and 6), sufficient time remains for cortisol concentrations to return to their appropriate postdexamethasone concentrations. More research is needed to determine the effects of a stressful event, such as ultrasonography, on dogs with chronic illness and hyperplastic adrenal glands.

<sup>a</sup>Cortrosyn, Organon Inc, West Orange, NJ.

<sup>b</sup>Cortisol [<sup>125</sup>I] RIA, Diagnostic Products Corp, Los Angeles, Calif.

<sup>c</sup>SIGMASTAT 2.0 for Windows, SPSS Inc, Chicago, Ill.

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