


STANDARD ARTICLE

Low-dose ACTH stimulation testing in dogs suspected of hypoadrenocorticism

Annabel Botsford^{1,4} | Ellen N. Behrend¹  | Robert J. Kempainen² | Philippe R. Gaillard³ | Frank Oprandy³ | Hollie P. Lee¹

¹Department of Clinical Sciences, Auburn University, Auburn, Alabama

²Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, Alabama

³Department of Mathematics and Statistics, Auburn University, Auburn, Alabama

⁴Atlantic Veterinary Internal Medicine and Oncology, Columbia, Maryland

Correspondence

Ellen N. Behrend, Department of Clinical Sciences, Auburn University, Auburn, AL 36849, USA.

Email: behreen@auburn.edu

Funding information

Interdepartmental Research Grants Program, College of Veterinary Medicine, Auburn University

Background: Low-dose ACTH stimulation testing would lower cost and may increase sensitivity for identification of partial ACTH deficiency.

Hypothesis: (1) The low-dose ACTH stimulation test will provide comparable results to the standard-dose ACTH stimulation test in dogs suspected of hypoadrenocorticism and (2) partial ACTH deficiency exists in dogs and can result in chronic, intermittent gastrointestinal signs.

Animals: Thirty-one client-owned dogs suspected of having hypoadrenocorticism.

Methods: Prospective study. Dogs suspected of having hypoadrenocorticism received 1 µg/kg cosyntropin IV for the first ACTH stimulation test; the second test was performed 4 h later and dogs received 5 µg/kg cosyntropin IV. Blood samples were obtained pre-ACTH and 1 hour post-ACTH for each dose (4 measurements total). Samples for endogenous ACTH measurement were obtained at the time of initial blood collection.

Results: No significant difference was observed in the basal cortisol concentration before administration of a 1 µg/kg versus before a 5 µg/kg dose of cosyntropin ($P = .544$). For dogs suspected of having hypoadrenocorticism, the ACTH-stimulated cortisol concentrations in response to both doses of ACTH were equivalent (90% confidence interval [CI], 80.5-97.2%; $P = .04$). No cases with partial ACTH deficiency were identified conclusively.

Conclusions and Clinical Importance: A 1 µg/kg dose of cosyntropin is equivalent to a 5 µg/kg dose of cosyntropin for screening dogs suspected of hypoadrenocorticism. The existence of partial ACTH deficiency was not identified in this small group of dogs.

KEYWORDS

Addison's, adrenal, cortisol, cosyntropin

1 | INTRODUCTION

Hypoadrenocorticism is an endocrine disorder that results in a deficiency of glucocorticoids and, in most cases, mineralocorticoids. Hypoadrenocorticism can be a result of primary adrenal failure or can be secondary to pituitary dysfunction and subsequent decreased or absent ACTH secretion.¹

Although the disease is fairly uncommon, testing for hypoadrenocorticism occurs relatively frequently because of the nonspecific signs and potential serious complications associated with a missed diagnosis. Definitive diagnosis is obtained by performing an ACTH stimulation test, an expensive procedure that may not be carried out because

of financial concerns of owners. In an effort to decrease cost associated with the diagnosis of hypoadrenocorticism, several laboratory, and imaging abnormalities have been investigated for their diagnostic utility. Variables that have been investigated include leukogram changes, sodium-to-potassium ratio, basal serum cortisol concentrations, serum aldosterone concentrations, cortisol-to-ACTH ratio, aldosterone-to-renin ratio, and size of the adrenal glands as measured by ultrasound examination.²⁻¹¹ Despite extensive research, definitive diagnosis of hypoadrenocorticism still cannot be reliably made without an ACTH stimulation test. In addition, performance of multiple screening tests in a patient ultimately may result in high cost and a delay in definitive diagnosis.

[Corrections added on 09 November 2018 after first publication: Pages 2, 3, and 5 have been updated to remove redundant text, to fix grammatical items, and update for clarity.]

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Use of a lower cosyntropin dose could decrease cost associated with ACTH stimulation testing. In healthy dogs, a 1 µg/kg dose of cosyntropin stimulates maximal adrenocortical cortisol secretion.¹² The lower 1 µg/kg dose has been evaluated in dogs suspected of or treated for hyperadrenocorticism, but, to our knowledge, has not been evaluated in dogs suspected of hypoadrenocorticism.

In humans, the spectrum of adrenal insufficiency ranges from overt adrenal crisis to subtle dysfunction in asymptomatic patients. Even with subtle dysfunction, patients may be at risk of developing acute adrenal insufficiency because their hypothalamic–pituitary–adrenal axis cannot react appropriately to stress.^{13,14} Therefore, identifying patients with subtle abnormalities is important. Subtle dysfunction can be caused by partial ACTH deficiency and is referred to as subclinical hypopituitarism. To our knowledge, a similar syndrome has not been documented in dogs, but the possibility of this phenomenon occurring in dogs has been discussed anecdotally. Use of a low-dose ACTH stimulation test may increase the test sensitivity for the diagnosis of hypoadrenocorticism and partial ACTH deficiency by detecting subtle changes in adrenocortical function. The dose of ACTH used in humans for diagnosis is as low as 1 µg per person.^{13,14}

Therefore, the objectives of our study were to (1) compare serum cortisol response when 1 and 5 µg/kg cosyntropin IV were administered to dogs suspected of hypoadrenocorticism and (2) investigate the possibility of partial ACTH deficiency in dogs with chronic, intermittent gastrointestinal signs. Our hypotheses were as follows: (1) the low-dose ACTH stimulation test would provide comparable results to the standard-dose ACTH stimulation test in dogs suspected of hypoadrenocorticism and (2) partial ACTH deficiency exists in dogs and can result in chronic, intermittent gastrointestinal signs.

2 | MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of Auburn University approved this study.

2.1 | Dogs suspected of hypoadrenocorticism

Client-owned dogs suspected of having hypoadrenocorticism were enrolled. Dogs were excluded if they were <1 year of age, <3 kg body weight, or had an adrenal tumor. Additional exclusion criteria included receiving PO or topical glucocorticoid treatment in the previous month, injectable glucocorticoid treatment in the previous 2 months, ketoconazole or progestin treatment in the previous month or etomidate or anesthesia within the previous 7 days. Dogs receiving trilostane or mitotane also were excluded.

Participants were enrolled at 2 university veterinary teaching hospitals and 1 specialty private practice. Hypoadrenocorticism was suspected based on history, physical examination findings, routine laboratory findings, or some combination of these.

Each dog underwent 2 ACTH stimulation tests in a single day as previously validated in healthy dogs.¹⁵ A dose of 1 µg/kg cosyntropin (Cortrosyn, Amphastar Pharmaceutical Inc., Rancho Cucamonga, CA) was administered IV (low-dose test). Blood was obtained before injection for measurement of serum cortisol and plasma endogenous ACTH

concentrations. An additional blood sample was obtained at 60 minutes postinjection (hour 1) for measurement of serum cortisol concentration. Three hours after collection of the post-ACTH sample (hour 4), a third blood sample was obtained for measurement of serum cortisol concentration and a second dose of cosyntropin (5 µg/kg IV) was administered (standard-dose test). An additional blood sample was obtained 1 hour later (hour 5) for measurement of serum cortisol concentration.

All samples for cortisol measurement were placed into plain collection tubes and centrifuged after clotting within 1 hour of collection. Serum was separated and stored at –20°C until analysis. Samples collected for measurement of endogenous ACTH were placed into vacuum tubes containing EDTA. Aprotinin (Trasylol, Aprotinin bovine lung lyophilized powder (Ultrapure), Affymetrix, Inc., Cleveland, OH) was added to the blood immediately (0.1 mL aprotinin [10,000 KIU/mL] per 2 mL blood).¹⁶ The sample then was mixed gently and centrifuged immediately. Plasma was removed immediately after centrifugation and stored in a plastic tube at –80°C until analysis.

An ACTH-stimulated serum cortisol concentration <2 µg/dL was considered diagnostic of hypoadrenocorticism, whereas an ACTH-stimulated serum cortisol concentration between 2 and 5 µg/dL was considered equivocal.^{4,17,18} Conversely, an ACTH-stimulated serum cortisol concentration >5 µg/dL excluded hypoadrenocorticism as a differential diagnosis.⁴ Partial ACTH deficiency was defined as an equivocal response to ACTH stimulation (ACTH-stimulated serum cortisol concentration between 2 and 5 µg/dL) when a dose of 5 µg/kg cosyntropin was administered in combination with a normal or subnormal plasma endogenous ACTH concentration. In the presence of decreased adrenal function, endogenous ACTH concentrations in normal dogs is expected to be above the reference range because of decreased negative feedback of cortisol to the pituitary gland. In addition, partial ACTH deficiency was suspected if disparate results were obtained between the low-dose and standard-dose ACTH stimulation tests.

2.2 | Preparation of cosyntropin

Cosyntropin was supplied as 250 µg of lyophilized powder in 2 mL vials. Each vial was reconstituted with 1.0 mL sterile saline solution to make a final concentration of 250 µg/mL in accordance with the manufacturer's directions. Unused cosyntropin remaining after reconstitution was frozen in 50 µg aliquots in plastic syringes until use or for no longer than 4 months.¹⁹ To administer the lower dose to small dogs, the cosyntropin was diluted to concentrations of 50 µg/mL and 10 µg/mL. To achieve a 50 µg/mL dilution, the original 1.0 mL (250 µg) of reconstituted cosyntropin was added to 4.0 mL sterile saline to achieve a final volume of 5.0 mL. To achieve a 10 µg/mL dilution, the original 1.0 mL (250 µg) of reconstituted cosyntropin was added to 24 mL of sterile saline to achieve a final volume of 25 mL. Diluted cosyntropin was stored frozen at –20°C in plastic syringes in 1 mL aliquots.²⁰ No cosyntropin was thawed and refrozen.

2.3 | Assay procedures

Serum cortisol concentrations were measured using a commercially available solid phase, competitive chemiluminescent enzyme immunoassay

(Immulite 1000 Cortisol Assay, Siemens HealthCare Diagnostics Products, Los Angeles, CA) that has been previously validated.²¹ The lower limit of analytical sensitivity is 0.5 µg/dL. For statistical purposes, results below the sensitivity of the assay were recorded as 0.3 µg/dL. Reported inter- and intra-assay coefficients of variation for the chemiluminescent enzyme immunoassay were >20% at 0.25 µg/dL, but ≤20% at values ranging from 0.5 to 25 µg/dL.²¹ The manufacturer reports cross-reactivity with prednisolone (49%), methylprednisolone (22%), corticosterone (8.6%), prednisone (5.9%), and negligible cross-reactivity with other steroids tested (<1%). Samples were analyzed in multiple batches and samples from each dog were run in a single batch. All samples were assayed in duplicate.

Plasma endogenous ACTH concentrations were measured using a commercially available and previously validated immunoradiometric (IRMA) assay (Scantibodies ACTH Immunoradiometric (IRMA) Assay, Scantibodies Laboratory, Inc. Santee, CA).²² The lower limit of analytical sensitivity was 5 pg/mL. For statistical purposes, results below the sensitivity of the assay were recorded as 2.5 pg/mL. Reported intra-assay and interassay coefficients of variation for the immunoradiometric assay are <15% and <19%.²² Samples were analyzed in a single batch and all samples were assayed in duplicate.

2.4 | Statistical analyses

Statistical analysis was performed using a commercial software package (SAS/STAT 13.2, SAS Institute, Cary, NC). For all tests, significance was set at the $P < .05$ level.

To compare baseline cortisol serum concentrations, a paired t test was performed. Pharmacologic equivalence of the ACTH doses was tested by determining if the 90% confidence interval (CI) for the ratio of the mean ACTH-stimulated cortisol concentration in response to each dose was fully contained within the equivalence region of 80%-125% for the ratio. It was determined that in an equivalence test of means using 2 one-sided tests on data from a 2-period cross-over design, a sample size of 32 would achieve 82% power at a 5% significance level when the true ratio of the means was 1.00, the coefficient of variation on the original unlogged scale was 0.30, and the equivalence limits of the mean ratio were 0.80 and 1.25.

3 | RESULTS

3.1 | Population

Thirty-two dogs suspected of hypoadrenocorticism were enrolled. After analysis, 1 dog was excluded because of extremely high serum cortisol concentrations, which did not allow for exact enumeration of the results and comparison of the response to both doses of cosyntropin. Of the 31 dogs included, 17 dogs were spayed female, 1 dog was an intact female, 11 dogs were neutered males, and 2 dogs were intact males. Breeds included were Labrador retriever ($n = 4$), Dachs-hund ($n = 4$), Australian shepherd ($n = 2$), Great Dane ($n = 2$), and 1 each of Pomeranian, Shih Tzu, Yorkshire terrier, German shepherd, Basset hound, Doberman, Boxer, Shetland sheepdog, Brussels Griffon, Bernese Mountain dog, Jack Russell terrier, American Staffordshire

terrier, Newfoundland, and Scottish terrier. Five mixed breed dogs were included. The median weight was 22.8 kg (range, 3.5-65 kg) and median age was 68 months (range, 15-154 months).

Presenting complaints included inappetence (15), vomiting (15), diarrhea (11), polyuria/polydipsia (2), weight loss (2), azotemia (1), hyperkalemia (1), hypoalbuminemia (1), and megaesophagus (1). Final diagnosis was not determined in 14 dogs (45%). In cases in which a diagnosis was obtained, final diagnoses included atypical hypoadrenocorticism (1), typical hypoadrenocorticism (1), chronic kidney disease (2), hemorrhagic enteritis (2), pancreatitis (2), inflammatory bowel disease (2), hepatocellular carcinoma (1), anaphylaxis (1), lymphoma (1), idiopathic megaesophagus (1), gastroenteritis (1), pyloric outflow tract obstruction (1), and hypercalcemia of unknown origin.¹

3.2 | Serum cortisol concentrations

Mean serum cortisol concentration before administration of 1 µg/kg of cosyntropin was 3.7 µg/dL, and mean serum cortisol concentration before administration of 5 µg/kg of cosyntropin was 3.4 µg/dL. No significant difference was detected between the basal serum cortisol concentrations ($P = .544$).

Mean serum cortisol concentration 1 hour after administration of 1 µg/kg of cosyntropin was 12.8 µg/dL, and mean serum cortisol concentration 1 hour after administration of 5 µg/kg of cosyntropin was 14.3 µg/dL. For dogs suspected of having hypoadrenocorticism, the ACTH-stimulated serum cortisol concentrations in response to both doses of ACTH were equivalent (90% CI, 80.5%-97.2%; $P = .04$; Figure 1).

Based on the diagnostic cut-off for hypoadrenocorticism of a post-ACTH serum cortisol concentration of <2 µg/dL, 2 dogs initially were diagnosed with hypoadrenocorticism. One dog was diagnosed with typical hypoadrenocorticism (post-ACTH serum cortisol concentration on the standard-dose test, 0.9 µg/dL) and 1 patient was diagnosed with atypical hypoadrenocorticism (post-ACTH serum cortisol concentration on the standard-dose test, 0.3 µg/dL). A third dog had an equivocal test for atypical hypoadrenocorticism (post-ACTH serum

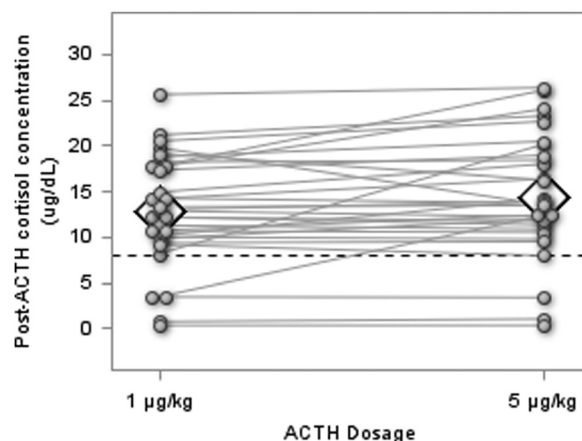


FIGURE 1 ACTH-stimulated cortisol concentration in dogs suspected of hypoadrenocorticism with low-dose and standard-dose ACTH stimulation test. Each point represents an individual dog. The diamonds represent the mean values of ACTH-stimulated cortisol. The lower limit of the reference range (8 µg/dL) for ACTH-stimulated cortisol concentration is represented by the dotted line

cortisol concentration on the standard-dose test 3.3 µg/dL and normal serum electrolyte concentrations).

Using a diagnostic cut-off of a post-ACTH serum cortisol concentration of <5 µg/dL, the interpretation of the low-dose and standard-dose tests differed in 1 dog. The post-ACTH serum cortisol concentration on the low-dose test was 3.4 µg/dL (in the equivocal range for hypoadrenocorticism), whereas the post-ACTH serum cortisol concentration on the standard-dose test was 12.3 µg/dL (not consistent with hypoadrenocorticism).

3.3 | Endogenous ACTH concentrations

The plasma endogenous ACTH concentrations ranged from 9 to 1429 pg/mL. The median plasma endogenous ACTH concentration was 21 pg/mL.

Partial ACTH deficiency was considered in 2 dogs. In the dog that had potential atypical hypoadrenocorticism with equivocal results after a standard-dose ACTH stimulation test (post-ACTH serum cortisol concentration of 3.3 µg/dL), the endogenous ACTH concentration was 73 pg/mL (reference range, 10–80 pg/mL). Glucocorticoid replacement therapy initially was prescribed, but was discontinued by the owners without adverse effect. In the patient with disparate ACTH stimulation test results, plasma endogenous ACTH concentration was 15 pg/mL. Treatment was not initiated, and results of both ACTH stimulation tests repeated 7 months after initial presentation were within the reference range.

4 | DISCUSSION

This study was performed to compare the results of a low-dose (1 µg/kg) ACTH stimulation test versus a standard-dose (5 µg/kg) ACTH stimulation test performed for the diagnosis of hypoadrenocorticism. Our results indicate that ACTH-stimulated serum cortisol concentrations obtained in response to both doses of ACTH were equivalent. Instead of including a patient population that consisted only of dogs proven to have hypoadrenocorticism, dogs suspected of having hypoadrenocorticism were included to simulate the population of dogs that are tested in practice. Therefore, the low-dose ACTH stimulation test can be used to confirm a diagnosis in dogs suspected of hypoadrenocorticism.

In our study population, partial ACTH deficiency was considered in 2 dogs. The first dog was a 5-year old intact male Shih Tzu initially evaluated for intermittent vomiting, inappetence and abdominal pain. Results of both ACTH stimulation tests in the presence of normal serum sodium and potassium concentrations were equivocal. The post-ACTH serum cortisol concentrations on the low-dose and standard-dose test were 3.4 and 3.3 µg/dL, respectively; the patient's plasma endogenous ACTH concentration was 73 pg/mL. In dogs with primary hypoadrenocorticism, plasma endogenous ACTH concentrations range from 200 to 5700 pg/mL as compared to a range of 5 to 9 pg/mL in dogs with secondary hypoadrenocorticism.²³ The endogenous ACTH assay utilized in the previous study is different than the assay utilized in our study, but, when the plasma endogenous ACTH concentration was <100 pg/mL, there was good agreement between the methods.²² Given the relatively low serum cortisol concentrations in response to ACTH in conjunction with a plasma endogenous ACTH concentration of 73 pg/mL (a result

inconsistent with classic primary hypoadrenocorticism), partial ACTH deficiency was considered possible. For treatment of hypoadrenocorticism, prednisone (0.34 mg/kg/day PO) was prescribed.

Despite initial subjective improvement, long-term follow-up by telephone call 2 years after presentation indicated that the patient continued to experience 3–4 episodes per year of vomiting, inappetence, and abdominal pain. Before the 2-year follow-up, prednisone therapy was discontinued, and clinical signs did not worsen. The continuation of clinical signs and knowledge that clinical signs did not worsen after prednisone was discontinued ultimately were not supportive of a diagnosis of hypoadrenocorticism. Unfortunately, repeat ACTH stimulation testing was not performed. This dog was similar to a group recently reported in which post-ACTH serum cortisol concentrations were >2.0 µg/dL, a commonly used cut-off for diagnosis of hypoadrenocorticism, but below the reference range.¹⁸ Our case further suggests that in dogs with such findings, another diagnosis, such as primary gastrointestinal disease, should be sought.

The second case in our study population with ACTH stimulation test results potentially consistent with partial ACTH deficiency was the dog that had discrepant results on the 2 tests. Possible reasons for the initial discrepancy between the results of the 2 ACTH stimulation tests include loss of potency of cosyntropin (because of poor or extended storage), use of an inappropriate cosyntropin dose or errors in administration or sample collection. Given that the storage and administration of cosyntropin and subsequent sample collection were directly supervised throughout the study period, these possibilities are considered unlikely. Presence of critical illness-related corticosteroid insufficiency²⁴ is unlikely because the dog was not critically ill at the time of testing. Partial ACTH deficiency and subtle adrenocortical deficiency that could only be detected with a low-dose ACTH stimulation test seems unlikely given resolution of clinical signs without treatment for hypoadrenocorticism and the results of the second set of tests.

Thus, we did not find evidence of partial ACTH deficiency. However, because only 31 dogs were included, further study is necessary before drawing more definitive conclusions.

If using the lower dose, attention must be paid to a few factors. First, the timing of collection of the sample for measurement of ACTH-stimulated serum cortisol concentration is crucial. If using a dose of 1 µg/kg, the duration of peak ACTH-stimulated serum cortisol concentrations is short. The post-ACTH serum sample must be collected at exactly 60 minutes postinjection.^{12,25} Second, we only examined IV use of the low dose. Therefore, IM injection of a low dose of cosyntropin cannot be recommended based on our data. Third, cosyntropin was diluted to achieve more accurate dosing on the low-dose test for small dogs. In concentrations as low as 0.5 µg/mL, diluted Cortrosyn[®] remains stable for at least 4 months when refrigerated in plastic containers.¹⁹ Other cosyntropin products should not be diluted and stored for use, because the stability of such products is unknown. Last, given that 1 dog had discrepant results between the 2 tests, if equivocal results are obtained on a low-dose ACTH stimulation test, a second test using a standard dose should be considered for further evaluation.

In conclusion, the use of a 1 µg/kg dosage of cosyntropin is considered equivalent to a 5 µg/kg dose of cosyntropin for screening and diagnosing dogs with clinical signs or laboratory abnormalities consistent with hypoadrenocorticism. Additional investigation is needed to

further define the potential existence of partial ACTH deficiency in veterinary medicine.

ACKNOWLEDGMENTS

The authors thank Drs. Cindy Ward and Alex Chisholm for help with recruitment of cases and Ms. Janeva Cole for aid in collection of samples. This project was completed at Auburn University in collaboration with the University of Georgia and Pet Emergency and Specialty Center in La Mesa, California. The study was supported by funds from the Interdepartmental Research Grants Program, College of Veterinary Medicine, Auburn University. An abstract was presented at the 2017 ACVIM Forum, National Harbor, MD.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Approval from the IACUC and the Client Research Review Committee of Auburn University.

ORCID

Ellen N. Behrend  <https://orcid.org/0000-0002-5010-2983>

REFERENCES

1. Scott-Moncrieff JCR. Hypoadrenocorticism. In: Feldman EC, Nelson RW, Reusch CE, et al., eds. *Canine and Feline Endocrinology*. Vol 4. St. Louis: Elsevier; 2015:485-520.
2. Adler JA, Drobatz KJ, Hess RS. Abnormalities of serum electrolyte concentrations in dogs with hypoadrenocorticism. *J Vet Intern Med*. 2007;21:1168-1173.
3. Baumstark ME, Sieber-Ruckstuhl NS, Muller C, et al. Evaluation of aldosterone concentrations in dogs with hypoadrenocorticism. *J Vet Intern Med*. 2014;28:154-159.
4. Bovens C, Tennant K, Reeve J, et al. Basal serum cortisol concentration as a screening test for hypoadrenocorticism in dogs. *J Vet Intern Med*. 2014;28:1541-1545.
5. Hoerauf A, Reusch C. Ultrasonographic evaluation of the adrenal glands in six dogs with hypoadrenocorticism. *J Am Anim Hosp Assoc*. 1999;35:214-218.
6. Javadi S, Galac S, Boer P, et al. Aldosterone-to-renin and cortisol-to-adrenocorticotropic hormone ratios in healthy dogs and dogs with primary hypoadrenocorticism. *J Vet Intern Med*. 2006;20:556-561.
7. Lathan P, Scott-Moncrieff JC, Wills RW. Use of the cortisol-to-ACTH ratio for diagnosis of primary hypoadrenocorticism in dogs. *J Vet Intern Med*. 2014;28:1546-1550.
8. Lennon EM, Boyle TE, Hutchins RG, et al. Use of basal serum or plasma cortisol concentrations to rule out a diagnosis of hypoadrenocorticism in dogs: 123 cases (2000-2005). *J Am Vet Med Assoc*. 2007;231:413-416.
9. Seth M, Drobatz KJ, Church DB, et al. White blood cell count and the sodium to potassium ratio to screen for hypoadrenocorticism in dogs. *J Vet Intern Med*. 2011;25:1351-1356.
10. Zeugswetter FK, Schwendenwein I. Diagnostic efficacy of the leukogram and the chemiluminometric ACTH measurement to diagnose canine hypoadrenocorticism. *Tierarztl Prax*. 2014;42:223-230.
11. Tschuor F, Zini E, Schellenberg S, et al. Evaluation of four methods used to measure plasma insulin-like growth factor 1 concentrations in healthy cats and cats with diabetes mellitus or other diseases. *Am J Vet Res*. 2012;73:1925-1931.
12. Martin LG, Behrend EN, Mealey KL, et al. Effect of low doses of cosyntropin on serum cortisol concentrations in clinically normal dogs. *Am J Vet Res*. 2007;68:555-560.
13. Reimondo G, Bovio S, Allasino B, et al. Secondary hypoadrenalism. *Pituitary*. 2008;11:147-154.
14. Fernandez-Rodriguez E, Bernabeu I, Andujar-Plata P, et al. Subclinical hypopituitarism. *Best Pract Res Clin Endocrinol Metab*. 2012;26:461-469.
15. Aldridge C, Behrend EN, Kempainen RJ, et al. Comparison of 2 doses for ACTH stimulation testing in dogs suspected of or treated for hyperadrenocorticism. *J Vet Intern Med*. 2016;30:1637-1641.
16. Kempainen RJ, Clark TP, Peterson ME. Preservative effect of aprotinin on canine plasma immunoreactive adrenocorticotropin concentrations. *Domest Anim Endocrinol*. 1994;11:355-362.
17. Gold AJ, Langlois DK, Refsal KR. Evaluation of basal serum or plasma cortisol concentrations for the diagnosis of hypoadrenocorticism in dogs. *J Vet Intern Med*. 2016;30:1798-1805.
18. Wakayama JA, Furrow E, Merkel LK, et al. A retrospective study of dogs with atypical hypoadrenocorticism: a diagnostic cut-off or continuum? *J Small Anim Pract*. 2017;58:365-371.
19. Frank LA, Oliver J. Comparison of serum cortisol concentrations in clinically normal dogs after administration of freshly reconstituted versus reconstituted and stored frozen cosyntropin. *J Am Vet Med Assoc*. 1998;212:1569-1571.
20. Dickstein G, Shechner C, Nicholson WE, et al. Adrenocorticotropin stimulation test: effects of basal cortisol level, time of day, and suggested new sensitive low dose test. *J Clin Endocrinol Metab*. 1991;72:773-778.
21. Singh AK, Jiang Y, White T, et al. Validation of nonradioactive chemiluminescent immunoassay methods for the analysis of thyroxine and cortisol in blood samples obtained from dogs, cats, and horses. *J Vet Diagn Invest*. 1997;9:261-268.
22. Martin LG, Groman RP, Fletcher DJ, et al. Pituitary-adrenal function in canine acute critical illness. *J Am Vet Med Assoc*. 2008;233:87-95.
23. Peterson ME, Kintzer PP, Kass PH. Pretreatment clinical and laboratory findings in dogs with hypoadrenocorticism: 225 cases (1979-1993). *J Am Vet Med Assoc*. 1996;208:85-91.
24. Martin LG. Critical illness-related corticosteroid insufficiency in small animals. *Vet Clin North Am Small Anim Pract*. 2011;41:767-782.
25. Kerl ME, Peterson ME, Wallace MS, et al. Evaluation of a low-dose synthetic adrenocorticotrophic hormone stimulation test in clinically normal dogs and dogs with naturally developing hyperadrenocorticism. *J Am Vet Med Assoc*. 1999;214:1497-1501.

How to cite this article: Botsford A, Behrend EN, Kempainen RJ, Gaillard PR, Oprandy F, Lee HP. Low-dose ACTH stimulation testing in dogs suspected of hypoadrenocorticism. *J Vet Intern Med*. 2018;32:1886-1890. <https://doi.org/10.1111/jvim.15256>