Effect of low doses of cosyntropin on serum cortisol concentrations in clinically normal dogs

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Objective—To determine the lowest of 5 doses of cosyntropin (1.0, 0.5, 0.1, 0.05, or 0.01 µg/kg) administered IV that stimulates maximal cortisol secretion in clinically normal dogs.

Animals—10 clinically normal dogs.

Procedures—5 dose-response experiments were performed in each of the dogs. Each dog received 5 doses of cosyntropin (1.0, 0.5, 0.1, 0.05, and 0.01 µg/kg) IV in random order (2-week interval between each dose). Serum samples for determination of cortisol concentrations were obtained before (baseline) and at 10, 20, 30, 40, 50, 60, 120, and 240 minutes after cosyntropin administration.

Results—Compared with baseline values, mean serum cortisol concentration in the study dogs increased significantly after administration of each of the 5 cosyntropin doses. Mean peak serum cortisol concentration was significantly lower after administration of 0.01, 0.05, and 0.1 µg of cosyntropin/kg, compared with findings after administration of 0.5 and 1.0 µg of cosyntropin/kg. After administration of 0.5 and 1.0 µg of cosyntropin/kg, mean peak serum cortisol concentration did not differ significantly; higher doses of cosyntropin resulted in more sustained increases in serum cortisol concentration, and peak response developed after a longer interval.

Conclusions and Clinical Relevance—Administration of cosyntropin IV at a dose of 0.5 µg/kg induced maximal cortisol secretion in healthy dogs. Serum cortisol concentration was reliably increased in all dogs after the administration of each of the 5 doses of cosyntropin. These data should be useful in subsequent studies to evaluate the hypothalamic-pituitary-adrenal axis in healthy and critically ill dogs. (*Am J Vet Res* 2007;68:555–560)

Adrenocorticotropin hormone stimulation tests are commonly used to evaluate adrenocortical function in dogs. The adrenocortical response of healthy dogs to IV administration of cosyntropin (synthetic ACTH) has been evaluated; findings after administration of several doses of synthetic ACTH (1, 5, or 10 µg/kg and 250 µg/dog) have been reported.[1-6] Results of those investigations indicated that all of the doses maximally stimulated the adrenal gland cortex. However, to the authors' knowledge, no studies have been performed to determine whether a dose of cosyntropin < 1 µg/kg would be sufficient to maximally stimulate the adrenal gland cortex in dogs.

Received August 4, 2006.
Accepted November 11, 2006.
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Funded by a grant from the Washington State University College of Veterinary Medicine Intramural Research Grant Program (Alder and Farrell Endowments).
Presented in part as a poster abstract at the 24th Annual American College of Veterinary Internal Medicine Forum, Louisville, June 2006.
The authors thank Dr. Ann Busch and Dr. Robert Kemppainen for assistance with the serum cortisol assays.
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Abbreviations

RAI	Relative adrenocortical insufficiency
HPA	Hypothalamic-pituitary-adrenal

Awareness of the minimal dose necessary to maximally stimulate the adrenal gland cortex in dogs may be crucial because of a syndrome that has been recognized in seriously ill human and veterinary patients.[7-15,a-e] The syndrome of RAI is characterized by an insufficient secretion of cortisol in relation to an increased demand during periods of stress, particularly in critical illnesses such as sepsis and septic shock,[7,11,16] and is usually defined by an inadequate response to exogenous ACTH.[7,12] A subnormal response indicates reduced functional integrity of the HPA axis and may lessen a patient's ability to cope with the stress of illness. In the setting of critical illness in humans, RAI appears to be transient and caused by the severe illness itself; adrenal gland function normalizes after recovery from illness. Typically, lifelong replacement of glucocorticoids is not needed.[7,8] The recognition of human and veterinary patients with RAI may be essential, not only because inadequate adrenal gland reserve appears to be associated with a poorer prognosis but also because administration of low doses of glucocorticoids may decrease morbidity and mortality rates, particularly among humans with sepsis.[8,13,14,17]

Adrenocorticotropin hormone stimulation testing is used to diagnose RAI in critically ill human patients. However, an apparently normal response to 250 µg of

ACTH/human patient does not rule out RAI[18,19] because injection of such a dose creates supraphysiologic serum ACTH concentrations (ie, more than 100-fold greater than normal endogenous stress-induced serum ACTH concentrations) that can override adrenal gland resistance to ACTH and result in an apparently normal cortisol response. Adrenal gland ACTH resistance is thought to be one of the mechanisms underlying RAI.[18,19] Thus, many physicians now use a more physiologic low-dose ACTH stimulation test involving 1 to 2 µg of ACTH (total dose)/patient, which better approximates circulating plasma ACTH concentrations associated with severe stress.[20-23] In healthy humans, low doses of synthetic ACTH (0.5 and 1 µg/patient) are sufficient to stimulate maximal release of cortisol from the adrenal glands.[24-28] Indeed, most critically ill patients with RAI respond in an apparently normally manner to the traditional high-dose ACTH stimulation test, but not to the physiologic low-dose test.[7,8,15,22] Thus, the low-dose ACTH stimulation test seems to be more sensitive than the standard-dose test for detection of RAI and reductions in adrenocortical reserve in humans.[15] The low-dose ACTH stimulation test is also capable of revealing mild adrenal gland insufficiency in humans receiving inhaled corticosteroids, and this insufficiency was not detected via the traditional high-dose ACTH stimulation test.[28]

In humans, physiologic endogenous plasma ACTH concentrations range from 100 to 300 pg/mL during severe stress, but are generally greater than 10,000 pg/mL after a stimulation test involving 250 µg of ACTH.[7,8] The reference range for endogenous plasma ACTH concentrations in dogs is approximately 10 to 80 pg/mL.[29] Physiologic endogenous ACTH concentrations are unknown for dogs during severe stress. However, Prittie et al[30] determined that endogenous plasma ACTH concentrations in a population of 20 critically ill dogs ranged from < 10 to 425 pg/mL. On the basis of the findings of Kerl et al,[3] the mean endogenous plasma ACTH concentration in dogs 30 minutes after IV administration of 5 µg of cosyntropin/kg was approximately 675 pg/mL.

The most commonly used protocols for ACTH stimulation testing in dogs involve IV administration of 250 µg of cosyntropin/dog or 5 µg of cosyntropin/kg to a maximum of 250 µg. Even though a 1 µg/kg dose of cosyntropin maximally stimulates the adrenal glands[2,3] and can be used to detect subtle changes in adrenocortical function,[2] it is currently not in widespread use. Because the traditional high-dose ACTH stimulation test (250 µg of cosyntropin/patient) administers a supraphysiologic dose of ACTH in humans, it is likely that this dose is supraphysiologic in dogs as well. Furthermore, because most dogs weigh less than adult humans, a dose of either 5 µg of cosyntropin/kg or 250 µg of cosyntropin/dog provides an even higher dose of ACTH (on a per-kilogram basis) in dogs than humans would receive if given 250 µg of cosyntropin. Therefore, we hypothesized that an IV dose of cosyntropin < 1.0 µg/kg would stimulate maximal cortisol secretion in clinically healthy dogs.

The purpose of the study reported here was to determine the lowest of 5 doses of cosyntropin (1.0, 0.5, 0.1, 0.05, or 0.01 µg/kg) administered IV that stimulates maximal cortisol secretion in clinically normal dogs. By gaining knowledge of how apparently normal adrenal glands respond to different low doses of cosyntropin, we believe that this will ultimately allow a better understanding of how illness affects the HPA axis and may also be useful in determining the most appropriate screening test for diagnosis of RAI in dogs.

Materials and Methods

Dogs—All aspects of the study were approved by the Institutional Animal Care and Use Committee at Washington State University. Ten clinically normal dogs owned by students, staff, or faculty of the Washington State University Veterinary Teaching Hospital were used. All dogs were enrolled with the informed consent of their owners. Dogs ranged in age from 1 to 8 years (mean age ± SD, 4.3 ± 2.8 years) and in weight from 17.6 to 42.3 kg (mean weight, 28.2 ± 7.9 kg). There were 6 female and 4 male dogs; all were spayed or neutered. Breeds represented included 5 mixed-breed dogs; 2 Labrador Retrievers; and 1 each of Plott Hound, Australian Cattle Dog, and Dalmatian. Dogs were deemed clinically normal on the basis of history; findings of physical examination; and results of a CBC, serum biochemical analysis, and urinalysis. Dogs receiving corticosteroids, etomidate, or ketoconazole were excluded from the study, as well as any dogs that had been treated with any of these medications within the previous month. Dogs with known or suspected adrenal gland disease (ie, hypoadrenocorticism or hyperadrenocorticism) were also excluded.

Procedures—Five dose-response experiments were performed. Each dog received 5 doses of cosyntropin[f] (1.0, 0.5, 0.1, 0.05, and 0.01 µg/kg) IV in random order with a 2-week washout period between each dose. All dose-response experiments were performed at the same time of day. The cosyntropin was administered as a bolus through a 16-gauge, 28-cm catheter placed in a jugular vein. Blood samples for determination of serum cortisol concentration were obtained through the jugular catheter. The catheter was flushed with 3 mL of saline (0.9% NaCl) solution containing heparin (10 U of heparin/mL of saline solution) after cosyntropin administration and after collection of each blood sample. Blood samples were obtained before (0 minutes; baseline) and at 10, 20, 30, 40, 50, 60, 120, and 240 minutes after cosyntropin administration.

Preparation of cosyntropin—Cosyntropin was supplied as 250 µg of lyophilized powder in 2-mL vials. Each vial of cosyntropin was reconstituted with 1.0 mL of sterile saline solution, in accordance with the manufacturer's directions, and was administered at this concentration (ie, 250 µg/mL) for the 1.0 µg/kg dose. All remaining doses (0.5, 0.1, 0.05, and 0.01 µg/kg) were administered after diluting the cosyntropin to 1 µg/mL as follows: the reconstituted contents of a single vial of cosyntropin were added to 249 mL of sterile saline solution to achieve a concentration of 1 µg of cosyntropin/mL. Until doses were administered, the solution was refrigerated in the plastic bottle that originally contained the sterile saline solution. Diluted cosyntropin

solutions remain fully stable (in concentrations as low as 0.5 µg/mL) for at least 4 months when refrigerated in plastic containers.[24] The diluted cosyntropin solution was stored in a refrigerator and used for the 10-week duration of the study.

Sample preparation and hormone assays—At the time of each sample collection from each dog, 3 mL of blood was obtained for determination of serum cortisol concentration and placed in a standard blood collection tube without anticoagulant. Samples were allowed to clot for 20 minutes prior to centrifugation. After centrifugation, the serum was removed, placed in a plastic tube, and frozen at –80°C until analysis. Serum cortisol concentration was measured by use of a radioimmunoassay[g] that had been previously validated for use in dogs.[2] The intra- and interassay coefficients of variation are 5.1% and 10.3%, respectively.[2] Four hundred fifty serum samples were assayed. Samples were assayed in multiple batches; however, samples from each dog were run in a single batch when possible. All samples were assayed in duplicate.

Statistical analysis—Results are reported as mean ± SD. To detect differences in serum cortisol concentration between doses and between time points, data were analyzed by use of a repeated-measures ANOVA in a commercial statistical computer program.[h] If statistical difference was identified, post hoc comparisons were made with a Bonferroni (all-pairwise) multiple comparison test. For all statistical analyses, a value of $P \leq 0.05$ was considered significant.

Results

Baseline serum cortisol concentrations in the 50 baseline samples obtained during the 5 cosyntropin response experiments ranged from 10 to 285 nmol/L (mean ± SD, 79.5 ± 18.7 nmol/L). Mean baseline serum cortisol concentrations in dogs prior to administration of each of the 5 doses of cosyntropin did not differ. Serum cortisol concentration increased significantly ($P < 0.001$) from baseline values after administration of each of the 5 cosyntropin doses. After administration of 0.01 µg of cosyntropin/kg, serum cortisol concentration peaked at 264% of baseline value (180.1 ± 57.3 nmol/L vs 68.2 ± 69.9 nmol/L) at 10 minutes and remained significantly greater than baseline value at 10, 20, and 30 minutes, before returning to baseline. After administration of 0.05 µg of cosyntropin/kg, serum cortisol concentration peaked at 268% of baseline value (244.9 ± 40.2 nmol/L vs 91.4 ± 53.7 nmol/L) at 20 minutes and was significantly greater than baseline value at 10, 20, 30, 40, and 50 minutes. After administration of 0.1 µg of cosyntropin/kg, serum cortisol concentration peaked at 274% of baseline value (289.7 ± 75.8 nmol/L vs 105.7 ± 83.0 nmol/L) at 30 minutes and was significantly greater than baseline value at 10, 20, 30, 40, 50, and 60 minutes. After administration of 0.5 µg of cosyntropin/kg, serum cortisol concentration peaked at 565% of baseline value (336.6 ± 53.5 nmol/L vs 59.6 ± 47.6 nmol/L) at 60 minutes. After administration of 1.0 µg of cosyntropin/kg, serum cortisol concentration peaked at 490% of baseline value (355.0 ± 56.9 nmol/L vs 72.4 ± 50.3 nmol/L) at 60 minutes. Mean serum cortisol concentrations were significantly increased, compared with baseline values, at 10, 20, 30, 40, 50, 60, and 120 minutes following cosyntropin administration at the 0.5 and 1.0 µg/kg doses (**Figure 1**).

On the basis of results of a 1-way ANOVA, mean peak serum cortisol concentration after administration of cosyntropin was significantly ($P < 0.001$) lower when doses of 0.01, 0.05, and 0.1 µg/kg were administered, compared with administration of doses of 0.5 and 1.0 µg/kg. Mean peak serum cortisol concentrations after administration of 0.5 and 1.0 µg of cosyntropin/kg did not differ significantly (**Figure 2**).

After administration of 0.01, 0.05, or 1.0 µg of cosyntropin/kg, the peak serum cortisol concentration at

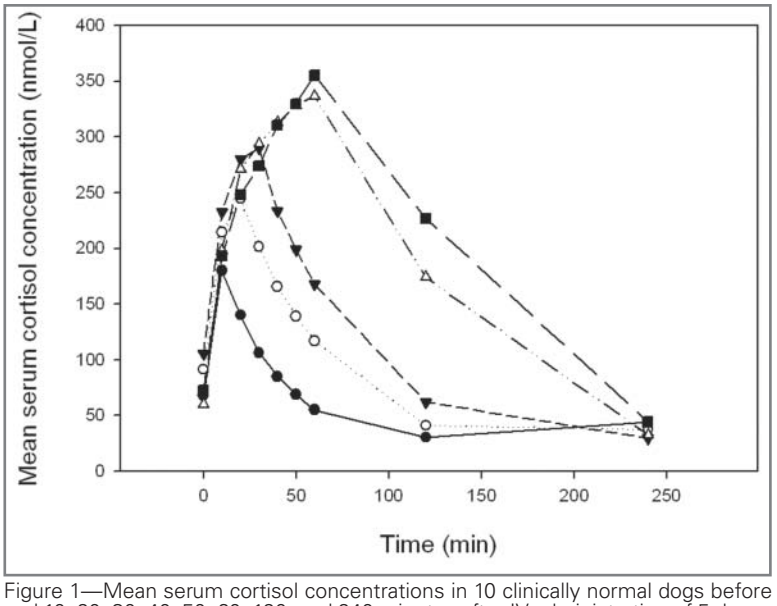

Figure 1—Mean serum cortisol concentrations in 10 clinically normal dogs before and 10, 20, 30, 40, 50, 60, 120, and 240 minutes after IV administration of 5 doses of cosyntropin (0.01 [closed circles], 0.05 [open circles], 0.1 [closed inverted triangles], 0.5 [open triangles], and 1.0 [squares] µg/kg).

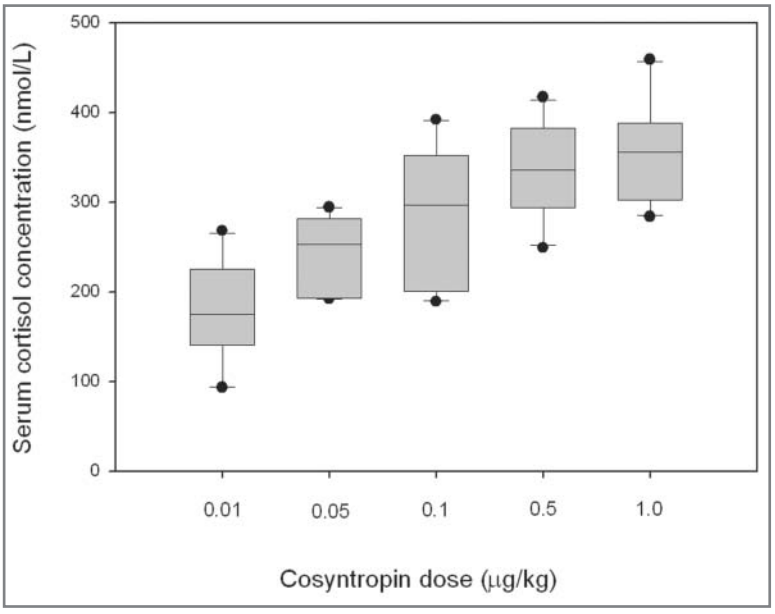

Figure 2—Peak serum cortisol concentrations in 10 clinically normal dogs after administration of the 5 doses of cosyntropin. Each box represents the interquartile (ie, 25th to 75th percentile) range, the horizontal line within the box represents the median value, the bars represent the 10th to 90th percentile, and the circles represent outlying datum points.

10, 20, and 60 minutes, respectively, was significantly different from that obtained at any other time point within the same dose group. Therefore, sampling times must be exact to identify serum cortisol concentrations that are representative of peak concentrations. After administration of 0.1 μg of cosyntropin/kg, the peak serum cortisol concentration was detected at 30 minutes; this value was significantly different from concentrations at all other time points, except for that at 20 minutes. This suggests that sample collection between 20 and 30 minutes after administration of 0.1 μg of cosyntropin/kg in dogs will reflect peak serum cortisol concentrations. After administration of 0.5 μg of cosyntropin/kg, the peak serum cortisol concentration was detected at 60 minutes; this value was significantly different from concentrations at all other time points, except for that at 40 and 50 minutes. Consequently, it appears that sample collection between 40 and 60 minutes after administration of 0.5 μg of cosyntropin/kg in dogs will reflect peak serum cortisol concentrations.

Discussion

Results of 2 previous studies[2,3] have indicated that a 1 μg/kg dose of cosyntropin administered IV results in maximal stimulation of the adrenal gland cortex in healthy dogs. Comparison of peak plasma cortisol concentration and time of peak response indicated that the results of those previous studies are consistent with the findings of the present investigation. In the study performed by Kerl et al,[3] 1.0 μg of cosyntropin/kg administered IV resulted in a mean ± SEM peak plasma cortisol concentration of 323 ± 21.3 nmol/L after 60 minutes. Higher doses of cosyntropin (5 and 10 μg/kg) induced peak serum cortisol responses later (90 minutes); however, the peak serum cortisol concentrations and areas under the cortisol response curve for these higher doses were not significantly different than the findings for the 1 μg/kg dose. The results of the present study are also similar to the findings of Kemppainen et al,[2] who administered 1.0 μg of cosyntropin/kg IV to healthy dogs and determined that the peak plasma cortisol concentration was similar to that attained in response to administration of 250 μg of cosyntropin/dog. In that study, IV administration of 1.0 μg of cosyntropin/kg resulted in mean ± SEM peak plasma cortisol concentration of 334.2 ± 16.8 nmol/L after 60 minutes. The similar findings among all 3 studies indicate that the 1.0 μg/kg dose of cosyntropin used in our investigation also resulted in maximal cortisol secretion in healthy dogs.

The results of our study in dogs indicated that mean peak serum cortisol concentrations after administration of the 0.01, 0.05, and 0.1 μg/kg doses of cosyntropin were significantly different from the peak concentration achieved after administration of the 1.0 μg/kg dose. The time to peak response was also decreased for the 3 lower doses, compared with the 1.0 μg/kg dose. However, mean peak serum cortisol concentration after cosyntropin administration and time to peak response did not differ significantly between the 0.5 and 1.0 μg/kg doses. Therefore, IV administration of cosyntropin at a dose of 0.5 μg/kg also resulted in maximal cortisol secretion in clinically healthy dogs. A dose of cosyntropin between 0.1 and 0.5 μg/kg may achieve maximal adrenal gland stimulation in healthy dogs, but further studies are required to determine the exact dose to do so.

In the dogs of the present study, higher doses of cosyntropin resulted in more sustained increases in serum cortisol concentration and a longer interval to peak response, ranging from a peak concentration at 10 minutes with return to baseline at 40 minutes after administration of 0.01 μg of cosyntropin/kg to a peak concentration at 60 minutes with return to baseline at

240 minutes after administration of 0.5 or 1.0 µg of cosyntropin/kg. This variability will be important if low-dose ACTH stimulation testing is performed in dogs because the current protocol involves collection of a blood sample 1 hour after cosyntropin administration, which would not adequately represent peak poststimulation serum cortisol concentrations if a dose of cosyntropin < 0.5 µg/kg is administered. For the 0.01 and 0.05 µg/kg doses, peak serum cortisol concentrations were detected at 10 and 20 minutes after cosyntropin administration, respectively. With these cosyntropin doses, the timing of blood sample collection must be exact; on analysis of samples collected prior to or after attainment of the peak cortisol concentration, serum cortisol concentrations would be significantly lower than the peak concentration. For the 0.1 and 0.5 µg/kg doses, peak serum cortisol concentrations were detected at 30 and 60 minutes after cosyntropin administration, respectively. With these cosyntropin doses, the timing of blood sample collection is not as crucial because analysis of samples collected within 10 minutes prior to attainment of peak cortisol concentration would also yield values reflective of the peak concentration. However, analysis of samples collected after attainment of the peak serum cortisol concentration would not detect the peak cortisol concentration. For the 1.0 µg/kg dose, blood sample collection at 60 minutes after cosyntropin administration will effectively identify peak serum cortisol concentrations, but the timing must be precise; sample collection before or after 60 minutes will yield lower serum cortisol concentrations that are not representative of maximum stimulation.

The design of the present study with regard to timing of sample collection, sample storage, and heparin usage should not have affected the results. For each dog in the study, all dose-response experiments were performed at the same time of day. On the basis of the findings of Kemppainen and Sartin,[31] even if experiments had been performed at various times, this factor is unlikely to have affected the response to cosyntropin. In healthy dogs, ACTH and cortisol are secreted episodically and not with circadian rhythmicity. Storage of serum samples at –20°C preserves cortisol concentrations.[32-34] Therefore, measurement of serum hormone concentrations after storage at –80°C (as performed in the present study) should reflect the concentrations at the time the blood samples were obtained. All dogs received 300 units of heparin during the study as a consequence of flushing the central venous catheter with saline solution containing heparin after cosyntropin administration and after collection of each blood sample. Heparin does not appear to affect cortisol secretion,[35] but may antagonize the actions of ACTH, especially with chronic use.[36,37] However, other sources report no interactions between heparin and ACTH.[38,39] Despite the inconsistent reports regarding the interactions of heparin with ACTH, it is unlikely that the heparin given to the dogs of the present study altered the results. In 2 other studies[2,3] in which 1 µg of cosyntropin/kg was given IV to healthy dogs, those investigators did not use heparin, and their results were similar to those of our study.

The results of the present study would appear to be useful in subsequent investigations to evaluate the HPA axis in critically ill dogs, specifically in the identification of dogs with RAI. The standard doses of synthetic ACTH (5 µg/kg and 250 µg/dog) currently used for ACTH stimulation testing are greater than that necessary to produce maximal adrenocortical stimulation in healthy dogs. Therefore, use of such doses may mask subtle decreases in adrenal gland reserve and hinder identification of dogs with RAI. Low-dose (0.5 µg/kg) ACTH stimulation testing should be compared with traditional-dose (5 µg/kg) ACTH stimulation testing in a population of critically ill dogs (ie, dogs with sepsis). If low-dose ACTH stimulation testing is found to be more sensitive for detecting insufficient adrenal gland function than traditional-dose ACTH stimulation testing, diagnostic screening for RAI could potentially be enhanced, and patient outcome may be improved if critically ill dogs with RAI were promptly identified and administered supplements of low doses of glucocorticoids.

In addition to its potential in diagnosis of RAI, low-dose ACTH stimulation testing may also be useful in screening dogs for mild hypoadrenocorticism. In healthy dogs 1 week after receiving prednisone IM at a dose of 2.2 mg/kg,[2] adrenocortical suppression was identified following administration of 1 µg of cosyntropin/kg but not following administration of 125 µg of cosyntropin/dog (7 to 21 µg/kg).[40]

With the recent substantial increase in the cost of cosyntropin, the ability to use low-dose ACTH stimulation testing increases the affordability of the test and, therefore, the frequency of use of this diagnostic test. Lower doses of cosyntropin are more cost-effective because multiple doses can be administered from one 250-µg vial without compromising the quality of test results. Cosyntropin, once reconstituted, can be stored in plastic syringes for as long as 4 months when refrigerated[23] or as long as 6 months at –20°C with no adverse effects on its bioactivity.[41] For instance, 1 vial of cosyntropin can be used to evaluate 20 dogs that each weighs 25 kg if each dog receives a dose of 0.5 µg/kg, which would result in a considerable reduction in the cost of the drug per dog. However, prior to application of low-dose ACTH stimulation testing to diagnose hypoadrenocorticism or hyperadrenocorticism in dogs, this diagnostic test would have to be properly validated in these patient populations.

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