Canine Pancreatic-Specific Lipase Concentrations in Clinically Healthy Dogs and Dogs with Naturally Occurring Hyperadrenocorticism

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Background: Specificity of canine pancreatic lipase immunoreactivity (cPLI) assays in dogs with hyperadrenocorticism (HAC) is unknown.

Hypothesis: Results of cPLI assays differ for clinically healthy dogs and dogs with HAC.

Animals: Seventeen healthy dogs and 20 dogs with HAC diagnosed by ACTH stimulation test results without evidence of clinical pancreatitis.

Methods: Dogs were enrolled between December 2009 and November 2010. Serum cPLI concentrations were determined by quantitative (Spec cPL test, SPEC) and semiquantitative (SNAP cPL test, SNAP) assays. Results were categorized as normal, equivocal, or abnormal (SPEC) or negative or positive (SNAP). Associations between group and cPLI were assessed using Fisher's exact test or the Mann–Whitney U-test. Spearman rank correlation coefficients (ρ) were determined for SNAP and SPEC results. Significance was set at $P < .05$.

Results: Spec cPL test concentrations were significantly ($P < .001$) higher in dogs with HAC (491.1 µg/L) than in healthy dogs (75.2 µg/L), with more abnormal SPEC results in HAC dogs ($P < .001$). There were more ($P = .002$) positive SNAP results in dogs with HAC (55%) than in healthy dogs (6%). SNAP and SPEC results were highly correlated ($\rho = 0.85$; $P < .001$).

Conclusions and Clinical Importance: Dogs with HAC had higher SPEC concentrations and more positive SNAP results than clinically healthy dogs with normal ACTH stimulation test results. Specificity of SPEC and SNAP assays in HAC dogs without clinical pancreatitis were 65 and 45%, respectively. Pending further study, SNAP and SPEC results should be interpreted cautiously in dogs with HAC to avoid false diagnosis of concurrent pancreatitis.

Key words: Cortisol; Cushings; Pancreatitis.

C anine hyperadrenocorticism (HAC) is a common and progressive endocrinopathy. Pancreatitis rarely has been reported as a cause of morbidity or mortality in dogs with HAC,[1,2] but HAC was identified as a risk factor for fatal pancreatitis in 1 study.[3] Clinicopathologic findings consistent with HAC include neutrophilia, lymphopenia, and thrombocytosis; increased serum alanine transferase and alkaline phosphatase (ALP) activities; and increased serum cholesterol, triglyceride, and glucose concentrations.[1] Many of these abnormalities also can occur secondary to pancreatitis. In addition, some biomarkers of pancreatitis are affected by steroid excess. These factors complicate accurate diagnosis of pancreatitis in dogs with HAC.

Abbreviations:

ALP	alkaline phosphatase
cPLI	canine pancreatic lipase immunoreactivity
HAC	hyperadrenocorticism
SNAP	SNAP cPL test
SPEC	Spec cPL test

Currently, serum canine pancreatic lipase immunoreactivity (cPLI) assays are considered the most specific serum biomarkers for the diagnosis of pancreatitis in dogs.[4,5] Reported specificities for the cPLI assays range from 71 to 100%.[4–7] Concentrations of cPLI can be estimated using a semiquantitative point-of-care test (SNAP)[a] or measured using a quantitative ELISA test (SPEC). The impact of naturally occurring HAC on cPLI assay results currently is unknown.

The purpose of this study was to compare SPEC and SNAP concentrations in clinically healthy dogs and dogs with HAC diagnosed by ACTH stimulation test results and that did not have clinical pancreatitis. The null hypothesis was that there would be no difference in SPEC concentrations or SNAP results between groups.

Materials and Methods

Study Population

Control Group. Privately owned, healthy dogs (n = 20) were recruited for the study by email to students, staff and faculty at the University of Tennessee College of Veterinary Medicine between October and November 2009. Dogs had to be ≥5 years of age, have received no medications other than routine parasite

prophylactics within the last 8 weeks, and have no clinical signs of disease or clinically relevant abnormalities on routine physical examination. Exclusion criteria were the presence of abnormalities on plasma biochemistry that persisted on reevaluation or abnormal post-ACTH stimulation test results. The experimental design was approved by the University of Tennessee Institutional Animal Care and Use Committee.

After enrollment in the study, dogs were fasted for 12 hours. Blood was collected at time 0 (t_0), 5 μg/kg ACTH[b] was administered IV, and a second blood sample was collected 1 hour post-ACTH stimulation (t_1). Plasma (t_0 only) and serum (t_0 and t_1) were separated from collected blood samples within 30 minutes by centrifugation at 8,450 g for 3 minutes. Plasma biochemistry panels and serum cortisol assays[c] were performed after enrollment of all dogs with HAC. Aliquots of t_0 serum were stored at $-80°C$, batched, and analyzed for SPEC concentrations and SNAP results with samples from dogs with HAC.

Dogs with HAC. Between December 2009 and November 2010, results from samples submitted to the University of Tennessee Clinical Endocrinology Service were reviewed to identify privately owned dogs with HAC for potential enrollment into the study. Initial exclusion criteria were presence of gross hemolysis and inadequate residual t_0 serum volume (<0.8 mL). Medical records were reviewed for cases evaluated at the University of Tennessee Veterinary Medical Center to obtain information regarding signalment, reasons for endocrine testing, clinical signs of disease (particularly relating to HAC or pancreatitis), medication history, and final diagnoses. For samples submitted from private clinics, one of the investigators (DIM) contacted the attending clinicians to obtain case information. A questionnaire (see Appendix) was used for initial case enrollment and data collection. This questionnaire did not provide referring veterinarians with information regarding purpose of the study. Referring veterinarians were queried regarding the presence of common clinical signs of HAC as well as clinical signs of hypoadrenocorticism, many of which also occur in patients with acute pancreatitis. This allowed assessment of clinical signs consistent with pancreatitis (eg, abdominal pain, vomiting, and diarrhea) while limiting potential recruitment and collection bias. Dogs with clinical signs of acute pancreatitis were excluded from the study.

Dogs were eligible for enrollment if they had a minimum of 1 common clinical sign of HAC or a minimum of 2 total clinical signs of HAC (as described in the ACVIM Consensus Statement regarding HAC)[8] as judged by their attending clinicians and confirmed on medical record review by one of the study authors (DIM or JCW), were receiving no medications at the time of the study (eg, glucocorticoids, op-DDD, trilostane, ketoconazole) that would be expected to alter ACTH stimulation test results, and had ACTH stimulation test results consistent with HAC based on laboratory reference intervals.

Residual t_0 serum from ACTH stimulation testing for dogs with HAC was separated into aliquots of 600 and 200 μL for SPEC and SNAP analysis, respectively, and stored at $-80°C$.[9,10]

Pancreatic-Specific Lipase Assays

Sera were shipped frozen to the Gastrointestinal Laboratory at Texas A&M University for SPEC analysis[11] using a monoclonal antibody and recombinant pancreatic lipase by an individual blinded to the results of other clinical data. Quantitative results were recorded for analysis. Results also were categorized by published diagnostic cut-offs as normal (0–200 μg/L), equivocal (201–399 μg/L), and abnormal (≥400 μg/L).[6]

The SNAP assays were performed by 2 individuals (DIM, JCW) in a batch in accordance with the manufacturer's guidelines. Samples were thawed for SNAP assay and labeled by an individual not associated with the study, so that the investigators performing the SNAP assays would be blinded to results of other assays at the time of assay performance. Results were categorized as negative or positive.

All samples were batched and assayed after study enrollment was completed to limit the impact of interassay variability on results.

Statistical Analysis

Descriptive statistics were calculated for quantitative variables by study group and analyzed for normality using the Kolmogorov–Smirnov test. Outliers noted on box-and-whisker plots were double-checked to eliminate data entry errors. Analytes with normal distribution for all groups were compared by Student's t-test (age) or paired Student's t-test (ACTH stimulation test results), whereas analytes with nonnormal distribution in at least 1 group were compared using the Mann–Whitney U-test (SPEC concentrations). Comparisons of categorical variables (SNAP results and SPEC concentrations by category) between groups were made using Fisher's exact test. Spearman rank correlation coefficients (ρ) between SPEC concentrations or SPEC diagnostic categories and SNAP results were determined. A value of $P < .05$ was considered significant for all tests. Statistical analyses were performed and figures created using commercially available statistical software packages.[d,e]

Results

Study Population

Twenty privately owned dogs initially were enrolled in the control group. There were no abnormalities on screening plasma biochemical profiles or ACTH stimulation test results for 16 of the dogs. One dog had increased ALP activity (198 U/L; reference range 15–164 U/L) on initial biochemical screening, but results were within the reference interval (103 U/L) on reevaluation and the dog was retained in the study population. Three dogs had abnormal post-ACTH stimulation serum cortisol concentrations and were excluded from further evaluation. Breeds for the remaining 17 dogs included Labrador Retriever (4), mixed breed (4), Border Collie (2), and 1 each of Chihuahua, Jack Russell Terrier, Rat Terrier, Doberman Pinscher, Mastiff, Staffordshire Terrier, and German Shorthaired Pointer. There were 7 spayed female dogs and 10 male dogs (9 neutered, 1 intact). Mean (±standard deviation [SD]) age was 7.6 (±2) years. No dog in the control group had evidence of clinical disease based on history or physical examination findings.

Twenty dogs with HAC were enrolled. Breeds included mixed breed (5), Dachshund (4), Australian Cattle Dog (3), Labrador Retriever (2), and 1 each of Beagle, Chihuahua, Havanese, Poodle, Shih Tzu, and Yorkshire Terrier. There were 14 spayed female dogs and 6 neutered males. Mean (±SD) age was 10.4 (±2.6) years. Common clinical signs[8] included polyuria and polydipsia (12), panting (9), polyphagia (5), endocrine alopecia (3), abdominal distention (2), muscle weakness (2), and hypertension (1). Other clinical signs included lethargy (2), urinary tract infection (2), and 1 each for pyoderma, calcium oxalate urolithiasis, and

weight gain. Dogs had a median of 2 clinical signs (range, 1–4). Diagnostic findings reported included proteinuria (4), adrenomegaly (4), and hypercholesterolemia (1). Eighteen of 20 dogs (90%) had increased serum ALP activities. One dog with polyuria and polydipsia and increased ALP activity had been receiving benazepril chronically for myocardial insufficiency at the time of evaluation.

Age, ACTH stimulation test results, and SPEC concentrations are summarized in Table 1. Healthy dogs were significantly ($P = .001$) younger than dogs with HAC. Consistent with inclusion criteria, ACTH stimulation test results were significantly ($P < .001$) higher for dogs with HAC than control dogs.

Pancreatic-Specific Lipase Assays

Results of the SPEC and SNAP assays are presented in Table 1 and Figures 1–3. Concentrations for SPEC were normally distributed in healthy dogs but not in dogs with HAC. SPEC concentrations were significantly higher in dogs with HAC than in healthy dogs ($P < .001$, Fig 1). The distribution of categorized SPEC results between groups was significantly different ($P < .001$, Fig 2), with significantly more dogs with HAC (7 dogs; 35%) with SPEC concentrations ≥400 µg/L than healthy dogs (0 dogs; 0%). There also were significantly ($P = .002$, Fig 3) more HAC dogs with positive SNAP results (9 dogs; 55%) than healthy dogs (1 dog; 6%). There was good correlation between SNAP assay results and SPEC concentrations ($\rho = 0.81$, $P < .001$).

Discussion

To the authors' knowledge, this study documents for the first time an association between naturally occurring HAC and increased cPLI assay results in dogs without clinical signs of pancreatitis. The goal of analyzing data for both SPEC and SNAP assays was to allow assessment of both potential association and diagnostic impact. Categorization of SPEC allowed determination of specificity using current clinical diagnostic cut-offs. In this study, SPEC concentrations were significantly higher in dogs with HAC than in healthy dogs. Using the established diagnostic cut-off value of 400 µg/L for pancreatitis, SPEC had a

specificity of 65% for dogs with HAC, and a specificity of 40% when a cut-off value of 200 µg/L was applied. There also were significantly more positive
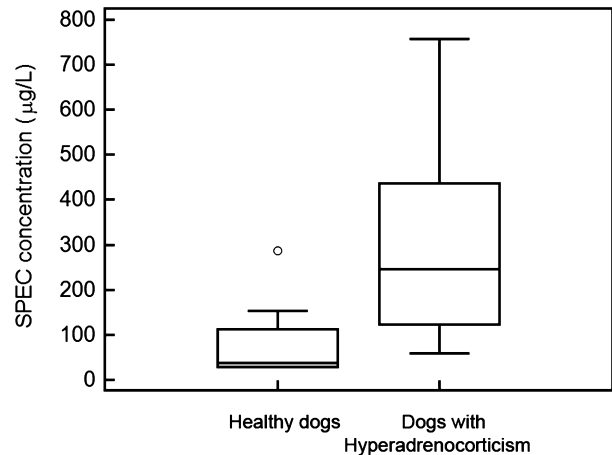
Fig 1. SPEC concentrations differed significantly ($P < .001$) between healthy dogs and dogs with hyperadrenocorticism. Note: One dog with hyperadrenocorticism, with a SPEC concentration of 4,248 µg/L, was censored from the graph to improve graph clarity.
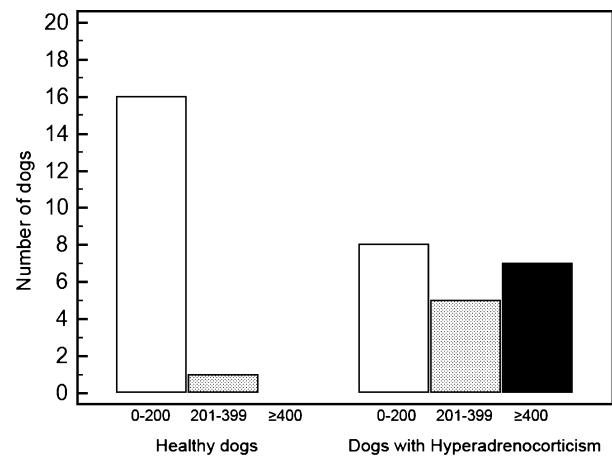

Fig 2. The distribution of SPEC concentrations differed significantly ($P < .001$) between healthy dogs and dogs with HAC when categorized using published criteria as normal (0–200 µg/L), questionable (200–399 µg/L), and abnormal (≥400 µg/L).[6]

Table 1. Age and results of ACTH stimulation tests and SPEC concentrations for healthy dogs and dogs with hyperadrenocorticism (HAC).

Analyte	Reference Interval	Healthy Dogs (n = 17)		Dogs with HAC (n = 20)	
		Mean	Median (Range)	Mean	Median (Range)
Age		7.6[a]	8.0 (5–13)	10.4	11.0 (6–14)
Baseline cortisol	2.0–58.8 ng/mL	29.8	17.6 (7.7–94.3)	57.7	55 (17–126)
Post-ACTH cortisol	65.0–174.6 ng/mL	119.2	117.5 (72–172)	367.4[b]	368.4 (218–501)
SPEC	≤200 µg/L	75.2	37.0 (29–287)	491.1[b]	248.5 (59–4,248)

[a]Dogs with HAC were significantly ($P = .001$) younger than healthy dogs.
[b]Results were significantly higher ($P < .001$) for dogs with HAC than for healthy dogs.
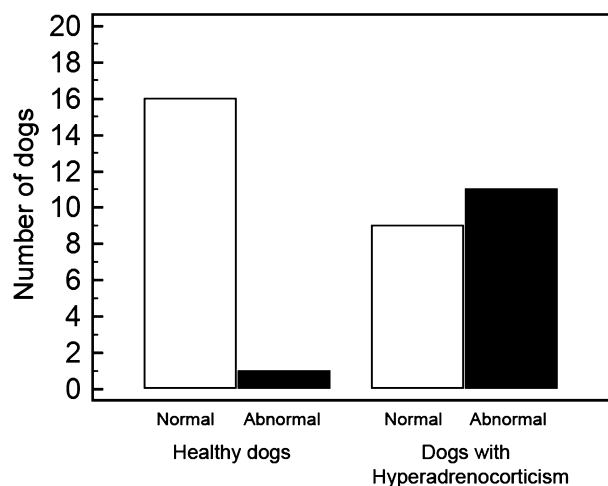
Fig 3. The distribution of SNAP results differed significantly ($P = .002$) between healthy dogs and dogs with hyperadrenocorticism.

SNAP results in dogs with HAC than in clinically healthy dogs, resulting in a specificity for the SNAP of 45% in dogs with naturally occurring HAC. Based on the results of this study, 35 and 55% of dogs with HAC that did not have clinical signs of pancreatitis could have been falsely categorized as having pancreatitis based on the SPEC and SNAP assays, respectively. Therefore, cPLI assays should be interpreted cautiously in dogs diagnosed with, or suspected of having, HAC that are being evaluated for potential concurrent clinical pancreatitis.

Increased canine cPLI concentrations currently are the most specific biomarkers for canine pancreatitis, with sensitivities ranging from 21 to 94% depending on disease severity.[4,6,12,f] Specificity of the SPEC assay for pancreatitis ranges from 66 to 100% depending on the study population and cut-off value used (200 and 400 μg/L).[4–7] In 1 report, sensitivities and specificities of the SPEC assay for clinical acute pancreatitis were 86.5–93.6% and 66.3–77.0%, respectively, using a cut-off value of 200 μg/L. Sensitivity and specificity were 71.7–77.8% and 80.5–88.0%, respectively, using the established diagnostic cut-off value of 400 μg/L.[6] The sensitivity and specificity of the SNAP were between 91.5–94.1% and 71.1–77.5%, respectively.[6]

Correlation between SPEC and SNAP has been reported to be 96%,[13] consistent with results of our study. Discordant results between the SPEC and SNAP assays were found in dogs with other final diagnoses, such as gastrointestinal disease, hepatopathy, or renal failure.[6] Authors of that report recommended further evaluation of the impact of renal disease and hepatopathy on cPLI results. Positive cPLI results have been reported in dogs without clinical signs of pancreatitis that had naturally occurring renal disease or hepatopathies.[6,7] Although results remained within the reference range, cPLI concentrations were higher in dogs with experimentally induced chronic kidney disease than in normal dogs.[10] SPEC

concentrations can be increased in dogs receiving potassium bromide, phenobarbital, or some other medications.[14,15] These increases could be because of drug-induced pancreatitis,[14–16] but alternate possibilities cannot be excluded.[16]

The effects of hypercortisolism on cPLI assays also have been evaluated. Prednisone (2.2 mg/kg/d) was administered PO to 6 young-adult female dogs with X-linked hereditary nephritis for 27 days with no influence on SPEC concentrations.[9] In contrast, both SNAP results and SPEC concentrations were significantly higher in dogs with naturally occurring HAC than in healthy dogs in this study. One explanation for the discordance in results is that this study had higher statistical power than the previous study.[9] Alternatively, the duration of steroid administration in the previous study could have been insufficient to result in altered SPEC concentrations. HAC develops insidiously, and it is likely that dogs with HAC had been experiencing steroid excess for >1 month at the time of diagnosis. The association between HAC and cPLI concentrations in this study could reflect vacuolar hepatopathy secondary to HAC. Approximately 30% of dogs with vacuolar hepatopathy have increased bile acids concentrations,[17] consistent with hepatic dysfunction. Abnormal cPLI concentrations have been reported in some dogs with hepatopathies.[6] Although hepatic biopsies were not obtained, 90% (18/20) of the dogs in this study had increased ALP activities. Because glomerular filtration rate is increased in dogs with HAC,[18] alterations in renal clearance are unlikely to have affected results of this study.

Because diagnostic evaluation was not standardized, some dogs with HAC could have had subclinical pancreatitis (ie, histologic inflammation of the pancreas without clinical signs of abdominal pain, vomiting, or diarrhea). Whether the chronic steroid excess present in dogs with HAC predisposes them to subclinical pancreatitis remains unresolved.[1] In 1 retrospective evaluation, 8/70 dogs (11.4%) with fatal acute pancreatitis had a previous diagnosis of HAC, consistent with a 4.3-fold increase in the risk of pancreatitis.[3] However, half of the dogs with HAC in that study had been receiving medical management for HAC before developing pancreatitis. Because of the retrospective nature of the study, the presence of confounding factors, including dyslipidemias, is unknown. Review of several long-term evaluations of dogs with naturally occurring HAC[2,19–22] did not identify a similar pattern of overrepresentation. Experimental studies in dogs also support a lack of association between glucocorticoid excess and pancreatitis.[23–29] Experimentally induced chronic steroid excess resulted in increased production and release of pancreatic enzymes into the duodenum without development of clinical pancreatitis.[23–29] Investigators were unsuccessful in inducing pancreatitis in an ex vivo perfused canine pancreas model after infusion of 200 and 400 mg methylprednisone,[26] and methylprednisone administration has been shown to have protective effects against hypovolemia-induced pancre-

atitis.[27] In addition, prednisone has been administered to dogs at 1.2 mg/kg/d PO or 4 mg/kg/d PO or IV for 14 days without development of clinical or histologic pancreatitis.[28] Finally, neither histologic nor clinical pancreatitis occurred in dogs receiving dexamethasone at 0.6 mg/kg/d SC for 21 days or receiving a 7-day, high-dose tapering protocol.[29] Abdominal ultrasound examination of all dogs by a board-certified radiologist would have been helpful to detect morphologic changes in the pancreas, although benign pancreatic hypertrophy can have a similar appearance to pancreatitis in some cases. Because histologic lesions can be highly localized,[30] collection of pancreatic biopsies would not have eliminated the possibility of occult inflammation. Conversely, the clinical relevance of mild histologic inflammation remains unclear.[7,31]

Alternately, the association between HAC and abnormal cPLI results may relate to underlying dyslipidemia. Both hypercholesterolemia and hypertriglyceridemia occur in dogs with HAC.[1] A syndrome known as benign pancreatic hyperenzymemia has been reported in people with hypercholesterolemia and hypertriglyceridemia.[32] People with benign pancreatic hyperenzymemia do not have pancreatic disease but have increased serum amylase, pancreatic isoamylase, and lipase activities. Obese dogs with marked postprandial hypertriglyceridemia have increased risk of increased cPLI concentrations, but not of developing clinical pancreatitis.[33] Benign hyperenzymemia may occur secondary to HAC or secondary to dyslipidemia in dogs with HAC. Unfortunately, plasma cholesterol and triglyceride concentrations were not measured in this study. Although no sample had gross lipemia, hypercholesterolemia can be present in nonlipemic plasma. It remains to be determined whether a type of benign hyperenzymemia occurs in dogs and what effect it would have on these serum enzyme activities.

There were some limitations to this study. Control dogs were significantly younger than dogs with HAC. In addition, the lack of differentiation of HAC as pituitary- or adrenal-dependent complicates application of results to populations with known distributions of pituitary- and adrenal-dependent disease. Adrenal adenocarcinoma could metastasize to the pancreas or alter blood flow, affecting cPLI results. Pituitary HAC results in increased serum ACTH concentrations and possibly other hormones. The effect of such hormone increases on cPLI assay results currently is unknown.

In conclusion, dogs with HAC but without clinical pancreatitis were more likely to have abnormal SPEC concentrations and SNAP results than clinically healthy dogs with normal ACTH stimulation test results. Based on this study, abnormal cPLI results should be interpreted with caution in dogs with HAC to avoid falsely diagnosing them with concurrent pancreatitis. Additional studies are warranted to determine the pathophysiology underlying this association.

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Appendix : Questionnaire for enrollment of dogs with HAC

Dear Dr. _____ :

We are currently performing a study utilizing leftover sera from dogs that received ACTH stimulation testing. Your clinic recently submitted an ACTH stimulation test to the UT Endocrinology Laboratory. We would greatly appreciate it if you would confirm the patient's signalment and answer a few brief questions. Provision of this information is anticipated to take no longer than 5 minutes.

Name: _____   Breed: _____

Gender: M / F (circle one)   Neutered?: Y / N (circle one)
Year of birth: _____

Please check (√) the box next to the disease you were evaluating with ACTH stimulation testing:
☐ Hyperadrenocorticism (Cushings)   ☐   Hypoadrenocorticism (Addisons)
   (initial diagnostic test)
☐ Hyperadrenocorticism (Treatment evaluation, i.e. Currently receiving Mitotane or
   trilostane)

Please check (√) the boxes next to all clinical signs present at time of ACTH stimulation test:
☐ Anorexia or hyporexia   ☐   Polyphagia
☐ Vomiting   ☐   Polyuria/polydipsia
☐ Diarrhea   ☐   Panting
☐ Abdominal pain   ☐   Other:_____

Please list any laboratory abnormalities present at time of ACTH stimulation test or send a copy of your blood work:
_____
_____

Please list any medications the patient was receiving at the time of ACTH stimulation test:
_____
_____

Additional comments?
_____
_____

Thank you so much for your time and effort to make this project possible. You may receive a follow up phone call regarding this study in the next couple of days.