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Plasma ACTH Precursors in Cats with Pituitary-Dependent Hyperadrenocorticism

G. Benchekroun, P. de Fornel-Thibaud, M. Dubord, O. Dossin, F. Fracassi, B. Rannou, F. Garnier, C. Maurey-Guenec, S. Daminet, and D. Rosenberg

Background: Diagnosis of pituitary-dependent hyperadrenocorticism (PDH) in cats is challenging because there is no specific diagnostic test.

Hypothesis/Objective: The determination of plasma ACTH precursor (POMC and pro-ACTH) concentration might facilitate the diagnosis of PDH in cats. The aim of the study was to evaluate prospectively the plasma concentrations of ACTH precursors in a small cohort of cats with PDH and to estimate the value of this approach for diagnosis.

Animals: Four groups of cats were included: group 1 (cats with PDH), group 2 (cats with diabetes mellitus but not hyperadrenocorticism (HAC)), group 3 (cats with diabetes mellitus and confirmed acromegaly but not HAC), and group 4 (healthy cats).

Methods: PDH diagnosis was based on clinical data, low-dose dexamethasone suppression test (LDDST), and adrenal and pituitary gland computed tomography (CT) scan. For groups 2, 3, and 4, hyperadrenocorticism was excluded by LDDST or urine cortisol:creatinine ratio (UCCR). An immunoluminometric assay was used to determine plasma concentrations of ACTH precursors in the 4 groups of cats.

Results: Group 1 contained 9 cats (enlarged pituitary gland in 7/9). Plasma ACTH precursor concentrations ranged from <53 to >1010 pmol/L with 8/9 concentrations $\geq$229 pmol/L. Groups 2, 3, and 4 included 13, 7, and 13 cats, respectively. Plasma ACTH precursor concentrations ranged from <53 to 96 pmol/L in group 2, <53 to 72 pmol/L in group 3, and <53 to 99 pmol/L in group 4.

Conclusion and Clinical Importance: High plasma concentration of ACTH precursors in cats (>100 pmol/L) is highly suggestive of PDH.

Key words: Corticotroph tumors; Cushing's disease; Feline; Pro-adrenocorticotropin.; Pro-opiomelanocortin.

Hyperadrenocorticism (HAC) in cats is a rare condition, with no more than 100 cases reported.[1–17] It is caused by a functional pituitary adenoma in 80% of cases.[1,16,17] About 50% of these pituitary tumors are microscopic, the others being large enough for visualization by computed tomography (CT) or magnetic resonance imaging (MRI).[1] Diagnosing HAC in cats is difficult. The most common clinical findings are poorly controlled diabetes mellitus (DM), associated with polyuria and polydipsia (PU/PD), weight loss, polyphagia, abdominal distension, muscle weakness, lethargy, and dermatologic abnormalities.[1–15] None of these clinical signs can be considered as specific, with the possible exception of the occasionally observed skin fragility syndrome.[1,12,16] Furthermore, PDH in cats is not the only condition characterized by a frequent association with DM and the common visualization of a pituitary tumor on imaging. These features are common to acromegaly in cats, and it might be difficult to distinguish between these 2 conditions in the absence of clinical signs specific to one of the 2 diseases.[18–21]

Corticotropic function tests have been validated for the diagnosis of HAC in cats, but lack specificity.[1] Urine cortisol:creatinine ratio (UCCR) and the low-dose dexamethasone suppression test (LDDST) are

Abbreviations:

BW	body weight
CT	computed tomography
$Cort_{8h}$	serum cortisol concentration 8 hours after low-dose dexamethasone administration
DM	diabetes mellitus
DSH	domestic shorthair
HAC	hyperadrenocorticism
IRMA	immunoradiometric assay
LDDST	low-dose dexamethasone suppression test
MRI	magnetic resonance imaging
PC1	prohormone convertase 1
PDH	pituitary-dependent hyperadrenocorticism
RIA	radioimmunoassay
UCCR	urine cortisol:creatinine ratio

From the Internal Medicine Unit, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France (Benchekroun, de Fornel-Thibaud, Maurey-Guenec, Rosenberg); and the Veterinary Anticancer Center, Maisons-Alfort, France (de Fornel-Thibaud); the Biochemistry Unit, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France (Dubord); the Department of Clinical Sciences and Clinical Research Unit, Ecole Nationale Vétérinaire de Toulouse, Toulouse, France (Dossin); the Department of Veterinary Medical Sciences, Università di Bologna, Ozzano dell'Emilia, Italia (Fracassi); the Biochemistry Unit, Vetagro Sup, Campus Vétérinaire de Lyon, Marcy l'Etoile, France (Rannou, Garnier); the Department of Small Animal Medicine, Ghent University, Merelbeke, Belgium (Daminet). *Part of this work was presented at the 2008 ACVIM Forum, Seattle, WA; at the annual ECVIM-CA Congress, in Ghent, Belgium, September 2008 (winner of the ESVE abstract award); and at the 2009 ACVIM Forum and Canadian Veterinary Medical Association Convention, Montreal, Quebec, Canada.*

Corresponding author: Dan Rosenberg, DVM, PhD. Internal Medicine Unit, Université Paris-Est, Ecole Nationale Vétérinaire d'Alfort, 7 avenue du Général de Gaulle, 94704, Maisons-Alfort, France; e-mail: drosenberg@vet-alfort.fr

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generally preferred to ACTH stimulation tests, which lack sensitivity.[1,8,22,23] The intravenous (IV) administration of 0.01 mg/kg dexamethasone is less frequently associated with feedback inhibition in healthy cats than in dogs. The LDDST is therefore generally based on the IV administration of 0.1 mg/kg dexamethasone in cats, to increase specificity.[24]

Deregulated secretion of adrenocorticotropic hormone (ACTH) by corticotroph tumor causes PDH. ACTH is derived from pro-opiomelanocortin (POMC) a high molecular weight polypeptide precursor. This precursor is first processed to pro-ACTH which is then cleaved to ACTH by the prohormone convertase 1 (PC1).[25] Plasma ACTH precursor (POMC and pro-ACTH) concentrations are high in large or aggressive pituitary corticotrophic tumors in both humans and dogs,[26–29] but no equivalent information is available for cats. The aim of this study was to describe plasma ACTH precursor (POMC and pro-ACTH) concentrations in cats with PDH and to estimate the utility of such determinations for the diagnosis of this condition, by comparing the results obtained with those for 3 other groups of cats: cats with DM but not HAC, cats with DM and confirmed acromegaly but not HAC, and healthy cats.

Materials and Methods

Clinical Cases

Cats were included prospectively in this study at the veterinary schools of Alfort and Toulouse (France), Ghent (Belgium), and Bologna (Italy). Four groups of cats were included. The cats in group 1 had PDH. Group 2 included cats with DM without HAC. Group 3 included cats with DM and confirmed acromegaly without HAC. Group 4 contained clinically healthy cats.

The inclusion criteria for group 1 were clinical findings consistent with HAC, confirmed by LDDST results, symmetric adrenal glands on contrast-enhanced CT scan, suggesting a pituitary origin, and availability of a pituitary contrast-enhanced CT scan for measurement of the pituitary gland height.

Cats were allocated to groups 2 and 3 after the diagnosis of DM on the basis of clinical findings, hyperglycemia, glycosuria, and serum fructosamine concentration above the reference interval, and exclusion of HAC. We excluded HAC on the basis of an absence of clinical findings consistent with it, with the exception of clinical signs of DM, and the results of UCCR or LDDST, the choice of the test used being left to the owner. Final inclusion in group 3 was based on insulin resistance (defined as poor clinical control and mean serum glucose concentration >300 mg/dL despite the use of insulin at a dose >1.5 units/kg/injection twice daily),[30] insulin-like growth factor-1 (IGF-1) concentrations consistent with acromegaly, and evidence of pituitary gland enlargement detected on contrast-enhanced CT scan. Cats were assigned to group 2 on the basis of data inconsistent with acromegaly, with at least 2 of the following criteria satisfied: no insulin resistance, IGF-1 within the reference interval, no CT scan or MRI performed, or no evidence of pituitary gland enlargement on CT or MRI.

Cats in group 4 were recruited from among staff members owned cats, based on history and clinical examination with no abnormality, no medication given within the past month, unremarkable results of serum biochemistry including serum glucose and fructosamine concentration within reference interval, and ruling out HAC by UCCR or LDDST, with the choice of test governed essentially by the lifestyle of the cat (living alone versus with other cats, limiting the simplicity of urine collection).

Laboratory Methods and Endocrine Tests

Serum and urine cortisol concentrations were determined with a kit,[a] previously validated for use in cats.[31,32] The LDDST was performed by injecting dexamethasone[b] IV at a dose of 0.1 mg/kg body weight (BW) and collecting blood at baseline and after 4 and 8 hours.[24,33,34] Reference values for serum cortisol concentration 8 hours after dexamethasone administration were $\leq$20 nmol/L. Serum cortisol concentration $\geq$40 nmol/L 8 hours after dexamethasone injection was considered consistent with HAC.[1]

Urine for UCCR determination was collected by the owners, at home, on the morning of the test.[22] Urine creatinine concentration was measured by an enzymatic method.[c] HAC was ruled out when UCCR values were below the upper limit of the reference interval ($4–36 \times 10^{-6}$).

Serum fructosamine concentration was determined with a colorometric assay.[d] The reference interval used was 200–370 μmol/L.

Total serum IGF-1 concentration was determined with a commercially available radioimmunoassay[e] validated for the cat, with reference values ranging from 208 to 443 ng/mL.[35] Serum IGF-1 concentration >1000 ng/mL in a diabetic cat was considered consistent with acromegaly.

Blood samples for the determination of ACTH and ACTH precursor concentrations were collected from the jugular vein into EDTA-coated tubes. The samples were immediately centrifuged at 4°C, 500 × *g* for 8 minutes, and the resulting plasma was transferred to plastic tubes and stored at −80°C until analysis.

Plasma ACTH concentration was measured with an immunoluminometric assay (ILMA)[f] previously used in cats.[7,36] The reference interval used was 8–93 pg/mL. We determined the POMC/pro-ACTH concentrations of plasma samples from cats with the OCTEIA POMC kit,[g] validated according to current procedures for bioanalytical analysis.[37] The calibration curve was obtained with 6 lyophilized human POMC calibrators diluted in a blank plasma matrix. The blank plasma matrix corresponded to a pooled collection of plasma samples from 10 healthy cats obtained 4 hours after the IV administration of dexamethasone phosphate at a dose of 1 mg/kg BW. Linearity was tested by diluting 1 sample with a high concentration of ACTH precursors in blank plasma matrix (dilution 1 : 2, 1 : 4, 1 : 8, and 1 : 16). The ratios of observed to expected values ranged from 100 to 109% (Table 1). The sensitivity of the assay, defined as the concentration corresponding to the mean plus 2 standard deviations for 5 replicates of the blank plasma matrix, was 53 pmol/L. Accuracy was determined by reconstituting 3 samples containing known concentrations of ACTH precursors with plasma matrix. Each sample was analyzed 5 times. Observed to expected ratios for spiking recovery ranged from 94 to 101% and accuracy was estimated as 98%. The intra-assay coefficient of variation obtained at 3 different samples with unknown concentrations measured 5 times each in a single assay ranged from 8 to 16% (Table 1). The interassay coefficient of variation obtained at 3 different samples with unknown concentrations measured in 5 consecutive assays ranged from 2 to 6%.

Computed Tomography

Food was withheld from the cats for 12 hours before CT scan. Anesthesia was induced by the IV administration of 6.5 mg/kg of propofol (1 g/100 mL),[h] and was maintained with inhaled isoflurane[i] and oxygen. Cats were placed in sternal recumbency and

Table 1. Partial validation of the OCTEIA POMC kit for use with cat plasma.

Sample	Concentrations		
	Measured (pmol/L)	Expected (pmol/L)	Measured/ Expected (%)
Linearity			
A	543	543	100
A/2	295	271	109
A/4	145	136	107
A/8	70	68	103
A/16	<53	<53	NA
Accuracy			
Sample 1	68 ± 1[a]	72	94
Sample 2	167 ± 3[a]	169	99
Sample 3	336 ± 11[a]	333	101
Precision			
Intra-assay (pmol/L)	CV% (n = 5)	Interassay (pmol/L)	CV% (n = 5)
78 ± 8[a]	10	57 ± 3[a]	6
223 ± 18[a]	8	231 ± 5[a]	2
728 ± 114[a]	16	351 ± 19[a]	5

[a]Mean ± SD.
CV, coefficient of variation; NA, not applicable.

a CT scan of the neurocranium between the external occipital protuberance and the cribiform plate was obtained before and 1 minute after the IV injection of contrast medium (2 mL/kg),[j] on a helical CT scanner.[k] Pituitary gland height was measured for each cat on the image with the largest cross-section of the pituitary gland. A pituitary height $\geq$3.7 mm was considered enlarged.[38] Contrast-enhanced CT scans were obtained for the cranial abdomen in the cats of group 1, 5–10 minutes after contrast administration for image acquisition of the pituitary gland region. Adrenal gland was subjectively considered symmetric in size in case of difference between the maximal diameter of the larger and the smaller gland <20%.

Statistical Analysis

Microsoft Excel[l] was used for descriptive statistics and data were described with medians and ranges. Plasma ACTH concentrations above the upper limit of quantification of the assay were reported as >1250 pg/mL. Plasma ACTH precursor concentrations below the analytical sensitivity of the assay were reported as <53 pmol/L and graphically plotted as = 53 pmol/L. Concentration above the upper limit of quantification of the assay was reported as >1,010 pmol/L and graphically plotted as = 1,010 pmol/L.

Plasma ACTH precursor concentrations for the 4 groups were compared by a Kruskal–Wallis one way analysis of variance test carried out with free software.[m] A P value < .05 was considered significant. Group to group testing was then assessed by Mann–Whitney rank sum tests. Bonferroni correction was used to adjust the type I error rate for multiple comparisons such that P value < .05/6 or P value < .008 was considered significant.

Results

The inclusion criteria were met by 9, 13, 7, and 13 cats in groups 1, 2, 3 and 4, respectively. There were 4 neutered males and 5 neutered females in group 1. One of these cases has already been described in a case report.[7] All were domestic shorthair (DSH) cats. Median age at the time of the diagnosis was 12 years (range: 10–15 years). Their clinical signs were PU/PD (n = 6), polyphagia (n =4), weight loss (n = 3), neuromuscular abnormalities such as weakness, abnormal behavior, neurologic deficits (n = 6), and dermatologic signs (n = 9). Five of these cats displayed skin fragility. Seven had poorly controlled DM. Median serum cortisol concentration 8 hours after low-dose dexamethasone administration ($Cort_{8h}$) was 152 nmol/L (range: 63–258 nmol/L). Plasma ACTH concentration was measured in 7 cats and ranged from 10 to >1250 pg/mL (median: 355 pg/mL), being within reference interval in one of the cat and above in the other 6. Seven cats had an enlarged pituitary gland, with a height of 6 mm to 27 mm (median: 8 mm). No pituitary gland abnormality was visible for 2 cats with typical clinical signs of HAC. Serum IGF-1 concentration was measured in 7 of the cats of this group (median: 85 ng/mL, range: 25–371 ng/mL). Serum fructosamine concentration was measured in 5 diabetic cats (median: 554 μmol/L, range: 411–652 μmol/L).

Group 2 included 7 male and 5 female DSH cats, and 1 male Russian blue cat, all neutered. Median age at inclusion was 13 years (range: 6–16 years). Clinical signs were: PU/PD (n = 13), polyphagia (n = 6), weight loss (n = 8), anorexia (n = 1), and plantigrade posture (n = 2). HAC was excluded on the basis of the LDDST in 6 cats (median $Cort_{8h}$: 9 mmol/L, range: <6–25) and UCCR in 7 cats (median: 6×10^{-6}, range: $1–8 \times 10^{-6}$). Pituitary gland was examined by CT scan only in one (with no abnormality). Median serum IGF-1 concentration was 457 ng/mL (range: 110–1489 ng/mL). Serum IGF-1 concentration was above reference interval (208–443 ng/mL) in 6 cats, <1000 ng/mL in 2cats (457 and 463 ng/mL), and >1000 ng/mL in the remaining 4 cats. Median serum fructosamine concentration was 597 μmol/L (range: 431–807 μmol/L).

Group 3 included 6 male cats and 1 female cat, all neutered. Five were DSH and the other 2 were Burmese cats. Median age at the time of diagnosis was 12 years (range: 11–14 years). Clinical signs were PU/PD (n = 7), polyphagia (n = 5), weakness (n = 2), weight gain (n = 2), plantigrade posture (n = 2), heart murmur (n = 2), prognathism (n = 2), increased interdental spacing (n = 1), stridor (n = 1), anorexia (n = 1), and weight loss (n = 1). Median IGF-1 concentration was 1094 ng/mL (range: 1000 to >1460 ng/mL). Median pituitary height was 5 mm (range: 4–8 mm). Median fructosamine concentration was 562 μmol/L (range: 429–787 μmol/L). HAC was excluded on the basis of UCCR in 6 cases (median: 7×10^{-6}, range: $2–30 \times 10^{-6}$) and LDDST in 1 case ($Cort_{8h}$: <6 nmol/L).

Group 4 comprised 9 neutered females, 1 neutered male, 1 intact male, and 2 intact females. Median age at the time of blood collection was 3 years (range: 1–11 years). All the cats in this group were DSH. HAC was excluded by UCCR in 10 cases (median: 6×10^{-6}, range: $5–9 \times 10^{-6}$) and by LDDST in 3

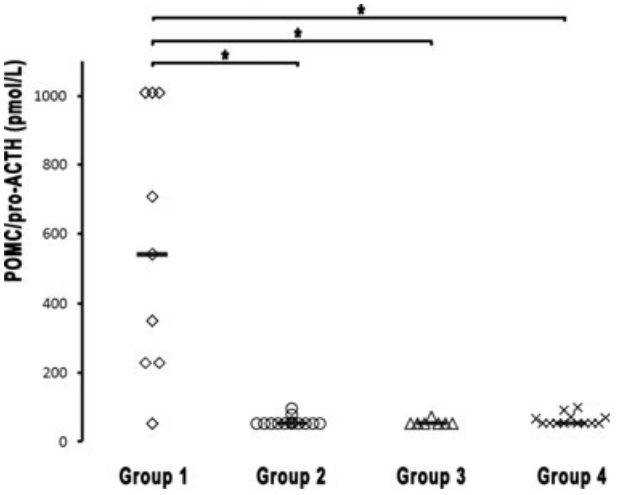

Fig 1. Pro-opiomelanocortin and pro-adrenocorticotropic hormone (POMC and pro-ACTH) concentrations in cats with PDH (group 1, diamonds), cats with DM with no demonstration of acromegaly (group 2, circles), cats with DM and acromegaly (group 3, triangles), and healthy cats (group 4, crosses). Measurements below the lower limit of quantification of the assay are assigned a concentration of 53 pmol/L, and measurement above the higher limit of quantification of the assay are assigned a concentration of 1010 pmol/L. Medians are indicated by horizontal lines. *Significantly different ($P < .008$).

cases ($Cort_{8h}$: <6 nmol/L). Median fructosamine concentration was 232 μmol/L (range: 179–267 μmol/L).

Plasma ACTH precursor (POMC and pro-ACTH) concentrations in group 1 ranged from <53 to >1010 pmol/L (median: 543 pmol/L) (Fig 1). In the 7 cats with an enlarged pituitary gland, concentration measurements ranged between 229 and >1010 pmol/L (median: 709 pmol/L). One of the cats without pituitary gland enlargement had an ACTH precursor concentration <53 pmol/L and the other had an ACTH precursor concentration of 229 pmol/L. In the other groups, plasma ACTH precursor concentrations ranged between <53 and 96 pmol/L (median: <53 pmol/L) for group 2, <53 and 72 pmol/L (median: <53 pmol/L) for group 3, and <53 and 99 pmol/L (median: <53 pmol/L) for group 4. The ACTH precursor concentration was <53 pmol/L in 10/13, 6/7, and 8/13 cats, respectively, in group 2, 3, and 4. Plasma ACTH precursor concentrations in the cats of group 1 were significantly higher than those in cats from groups 2, 3, or 4 ($P < .001$, $P = .004$, and $P = .003$, respectively). Plasma ACTH precursor concentrations did not differ significantly between the cats of groups 2, 3, and 4.

Discussion

In this study, we prospectively compared plasma ACTH precursor (POMC and pro-ACTH) concentrations in cats with PDH, DM, DM with acromegaly and healthy cats. PDH in cats is a rare disease and the largest survey of this disease to date included only 8 cases.[17] Despite the inclusion of cats from 4 different referral centers, we were able to include only 9 cases prospectively, making it difficult to estimate the sensitivity of the tool developed in this study.

PDH diagnosis is challenging in cats because of its vague clinical presentation and the inadequacies of the available diagnosis tools. In this study, 8/9 cats with PDH have a plasma ACTH precursor concentration $\geq$229 pmol/L, whereas none of the healthy cats or cats with DM with or without acromegaly had plasma ACTH precursor concentrations >99 pmol/L. These results suggest that corticotropic tumors in cats are associated with high plasma concentrations of ACTH precursors. In humans, it is assumed that the impairment of POMC processing in less differentiated pituitary tumors derived from corticotroph cells leads to an increase in the secretion of ACTH precursors into the plasma.[26,27,39] In dogs, high plasma ACTH precursor concentrations is correlated with the size of the pituitary tumor.[28,29] Plasma concentrations of ACTH precursors are high in 13/14 dogs with a large pituitary tumor (pituitary gland height of 5–22 mm).[29] Similar results were obtained in this study, with the recording of high plasma ACTH precursor concentrations in all 7 cats with an enlarged pituitary gland (6–27 mm in height). Plasma ACTH precursor concentrations above the upper limit of the range of values obtained for the control group were also found in 1/2 cats with PDH and no visible pituitary gland enlargement. ACTH precursors are rarely present at detectable concentrations in the plasma of dogs with PDH, but no enlargement of the pituitary gland.[29] This observation is reminiscent of another case with no enlargement of the pituitary gland (pituitary gland height: 2.9 mm) reported in a previous study focusing on hypophysectomy in cats with PDH.[8] A high plasma ACTH precursor concentration was indirectly suspected, based on comparisons of plasma ACTH concentrations determined by radioimmuoassay (RIA), which should theoretically detect both ACTH and ACTH precursors, and by an immunoradiometric assay (IRMA), which was more specific for ACTH. Histologic examination showed the differentiation of the pituitary gland of this cat to be similar to that in other hypophysectomized cats in the survey.[8]

Plasma ACTH concentration was measured in 7/9 cats with PDH with a commercialized ILMA claimed by the manufacturer as specific for ACTH concentration determination in humans. Indeed, these measurements appeared poorly correlated with plasma ACTH precursors concentrations measures in the same cats. Comparison of plasma ACTH concentrations RIA and ILMA to evaluate indirectly ACTH precursors concentrations was not conducted in this study. One PDH cat had ACTH concentration within reference interval. To date, very little is known about ACTH measurement in cats with HAC. In dogs, plasma ACTH concentration is within reference interval in 58/91 dogs with PDH.[40]

Hypophysectomy is rarely performed at our referral centers and postmortem examination was declined in

most of the PDH cases. Consequently, the main limitation of this study is the lack of histologic examination of the pituitary gland of the cats with PDH or acromegaly included. In addition to providing information about differentiation status, immunohistologic characterization of the pituitary tumors would have made it possible to confirm the corticotropic or somatotropic nature of the tumor, and the correct allocation of the cats to groups 1 and 3. Despite this limitation, the assignment of cats to these 2 groups was probably accurate. We used the most specific criteria possible to limit the risk of misallocation in the 2 groups. In 7/9 cats in group 1, the association of clinical signs compatible with HAC, the absence of feedback inhibition after LDDST with a dose of dexamethasone (0.1 mg/kg) limiting the false-positive rate and the detection of pituitary gland enlargement, provided strong evidence of PDH.[1] Serum IGF-1 concentration was measured in 5 of these 7 cats and was within the reference interval, confirming the low probability of acromegaly. PDH in cats may be associated with the absence of visible pituitary gland enlargement on CT scan, as was the case for 2 animals in this study.[1,8] Both cats concerned had a serum IGF-1 concentration within the reference interval. One had a potbellied appearance and acquired skin fragility, which is rarely observed in contexts other than HAC in cats. It has occasionally been reported after the use of progestagens, or in the contexts of DM without documented HAC, disseminated histoplasmosis, and various biliary and hepatic diseases.[41–44] Routine biochemistry and hematologic examination, and complete ultrasound examination of the abdomen identified none of these features in this cat, other than DM. The 2nd cat was the only animal in group 1 to have a plasma ACTH precursor concentration below the lower limit of quantification of the assay. The clinical signs of this cat were suggestive of HAC (abdominal distension, thin and inelastic skin with alopecia, weight loss, and poorly controlled DM). It had an unsuppressed cortisol concentration, 4 and 8 hours after LDDST (320 nmol/L and 180 nmol/L, respectively). In dogs, the risk for any corticotropic function test, including the LDDST, of false-positive results suggesting HAC is frequently highlighted in reference textbooks and review manuscripts, especially when DM is present.[45,46] However, this risk has rarely been investigated in cats. In 1 recent study, suppression was systematically observed after LDDST with 0.1 mg/kg dexamethasone in 20 cats with DM, regardless of the quality of glycaemia control, whereas no suppression was observed in 2 additional cats subsequently diagnosed with HAC.[34] Given these findings and the clinical features of this cat, the probability of misclassification to group 1 appears low.

In this study, cats were assigned to group 3 on the basis of a combination of insulin resistance, high IGF-1 concentration, and pituitary gland enlargement. Cases of acromegaly lacking at least one of these criteria have already been described in cats and it is therefore possible that some cases of acromegaly were missed with this strategy.[35,47,48] Conversely, none of these criteria including IGF-1 concentration >1000 ng/mL can be considered specific of acromegaly separately, but the probability of incorrectly including cats without acromegaly in group 3 appears low with these 3 robust criteria taken together.[35,48,49] The high ratio of males to females (6/7 cats) is consistent with published findings.[35,47] Moreover, although the clinical presentation of acromegaly in cats is polymorphic, some features are reasonably specific. In 4/7 cats, at least one of the following features, weight gain despite insulin resistance, morphologic changes, or stridor, was present increasing even more our confidence that the cats had been correctly assigned.

One cat in group 3 and 1 cat in group 2 was anorexic. Although described in acromegalic and diabetic cats,[50,51] anorexia is usually a sign of complicated diabetes or concurrent disease. The 2 cats had uncomplicated diabetes mellitus and no concurrent disease was identified.

Acromegaly in cats is underdiagnosed.[35] Neither insulin resistance, IGF-1 concentration above our diagnostic cut-off, nor pituitary gland enlargement can be considered perfectly sensitive for acromegaly diagnosis.[35,48] Thus, it is possible that acromegalic cats were included in group 2. This is particularly true for the 4 cats with serum IGF-1 concentrations >1,000 ng/mL. The serum IGF-1 concentrations of cats with DM without acromegaly increase with treatment duration, but generally remain within the reference interval, which was not the case for these 4 cats. Three of them had poorly controlled DM on enrollment, but did not meet the criteria used here for insulin resistance. The owners of these 4 cats refused permission for CT scans of the pituitary glands of the cats, which were thus assigned to group 2. Bearing these doubts about the correct allocation of these 4 cats in mind, the clear difference in ACTH precursor concentration between cats with PDH and non HAC cats with DM with or without demonstrated acromegaly (groups 2 and 3) is likely to be useful to clinicians, as HAC in cats is often diagnosed in a context of DM, suspected acromegaly, or both.[1–3,21]

The use of OCTEIA POMC kit in cat was only partially validated in this study. Precision was determined with minimal number of replicates in this context of scarcity of samples from cats containing detectable concentrations of ACTH precursors.[37] The size and format (eg, median age at 3 years) of group 4 didn't allow the determination of a reference interval. Interference (eg, hemolysis, lipemia, hyperbilirubinemia) and stability at different conditions of storage were not tested either. We used stringent preanalytical conditions: blood centrifugation at 4°C, immediate freezing of plasma until analysis, by analogy with the requirements for plasma ACTH concentration measurement.[52] We are aware of no published data relating to the stability of ACTH precursors in humans. In dogs, preliminary results have indicated that simple sampling and shipping procedures can be used.[n] The commercialization of the OCTEIA POMC kit has been stopped recently limiting the possibility of using the

results of this study in clinical field. However, antibodies on which this commercial assay was based are still available in the lab where they were developed.[o] Validation of a new assay developed with the same antibodies appears possible in theory.

In conclusion, ACTH precursor concentration $\geq$229 pmol/L was measured in 8/9 cats with PDH. No plasma POMC/pro-ACTH concentration $\geq$100 pmol/L was found in a relatively large number of cats without PDH (cats with DM with or without acromegaly and healthy cats). Subject to a new validation of an assay allowing ACTH precursor concentration measurement and confirmation in a larger cohort of cats with PDH, the use of this kind of tool may be of value for the diagnosis of PDH in cats and for the general diagnostic approach to DM in cats.

Footnotes

[a] Immulite cortisol, Siemens Medical Solutions Diagnostics, Los Angeles, CA
[b] Dexadreson, dexamethasone phosphate, 2 mg/mL, MSD Animal Health, Beaucouzé, France
[c] Konelab creatinine (Enzymatic), Thermo Fisher Scientific, Vantaa, Finland
[d] Fructosamine, Roche, Basel, Switzerland
[e] Cambridge Specialist Laboratories Services Ltd, Cambridge, UK
[f] Immulite ACTH, Siemens Medical Solutions Diagnostics
[g] OCTEIA POMC, Immunodiagnostic Systems Limited, Boldon, Tyne and Wear, UK
[h] Rapinovet, Schering-Plough Vétérinaire, Levallois-Perret, France
[i] Isoflurane aerrane, Baxter SAS, Maurepas, France
[j] Télébrix 35, sodium and meglumine ioxitalamate, 350 mg of iodine/mL. Laboratoire Guerbet, Roissy-Charles-de-Gaulle, France
[k] Scanner Hispeed CT/e Plus. General Electric Medical Systems, Milwaukee, WI
[l] Microsoft Office Excel 2003, Microsoft Corporation, Redmond, WA
[m] VassarStats, http://faculty.vassar.edu/lowry/VassarStats.html
[n] de Fornel-Thibaud P, Granger N, Rosenberg D. Effect of aprotinin, centrifugation, and storage temperatures on stability of canine plasma adrenocorticotrophic hormone precursors before analysis, J Vet Intern Med 2005;19:423 (abstract)
[o] Professor Anne White, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK

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