ACTH stimulation test in the captive cheetah (*Acinonyx jubatus*)

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ABSTRACT

Serum cortisol response was assessed in 8 captive cheetahs, of varying ages, after the intravenous administration of 500 μg of tetracosactide (Synacthen Depot®, Novartis, Kempton Park) while maintained under general anaesthesia. In addition, 8 cheetahs were anaesthetised and given an equal volume of saline in order to establish baseline cortisol concentrations at similar stages of anaesthesia. A significant difference in the median cortisol concentration measured over time was found following ACTH administration in the ACTH group ($P < 0.001$). There was no difference between the median cortisol concentrations in the ACTH group at time-points 120, 150 and 180 min after ACTH stimulation ($P = 0.867$). Thus it appears appropriate to collect serum 120 to 180 min after tetracosactide administration to assess maximal stimulation of the adrenal in the cheetah. No statistically significant rise was seen in the anaesthetised control group following the injection of saline ($P = 0.238$).

Key words: adrenal function, cortisol, Synacthen Depot®, tetracosactide.

Köster L S, Schoeman J P, Meltzer D G A **ACTH stimulation test in the captive cheetah (*Acinonyx jubatus*).** *Journal of the South African Veterinary Association* (2007) 78(3): 133–136. Veterinary Wildlife Unit, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Captive cheetahs (*Acinonyx jubatus*) have a high incidence of veno-occlusive disease, chronic gastritis, and glomerulosclerosis[8]. *Helicobacter* has been isolated in both free-ranging and captive cheetah, but gastritis has not been diagnosed in free-ranging animals[17]. It appears that *Helicobacter* infection alone does not cause this disease[17]. It has been proposed that stress could play a major role in the development of helicobacteriosis associated gastritis in captive cheetah[6,20]. Adrenal cortical hypertrophy has been shown to be associated with gastritis in captive cheetahs, thus assessing stress may be the key to understanding the pathogenesis of gastritis in these animals[9]. Captive cheetahs have significantly higher baseline faecal corticoid concentrations and adrenal cortical hypertrophy compared to free-ranging animals[19].

The adrenocorticotrophic hormone (ACTH) stimulation test is widely accepted for the evaluation of adrenocortical function in humans, dogs and cats[13]. There are various formulations of ACTH available world-wide, including natural ACTH gel and synthetic formulations. Synthetic formulations include cosyntropin (USA) and tetracosactrin (Europe). The synthetic formulation available in South Africa is a long-acting corticotrophic preparation, known as tetracosactide (Synacthen Depot®, Novartis, Kempton Park), in the form of a suspension in which the active substance is adsorbed onto an inorganic zinc complex. The synthetic formulations all share the first 24 amino acid sequence found in naturally occurring ACTH[4]. The temporal association between cortisol concentration increasing and peaking after an ACTH gel given intramuscularly and a synthetic formulation (cosyntropin) given intravenously was shown to be broadly similar, although the peak was reached slightly later in cats receiving the gel formulation[10]. A comparative study did not demonstrate a statistically significant difference in adrenocortical response between the cosyntropin and tetracosactrin formulations[12].

A standardised protocol which produces a consistent stimulation of the endocrine gland must be used for the ACTH stimulation test. The dose of ACTH and the time interval between the collection of the baseline and post-stimulation blood samples must be adequate to ensure hormone concentrations are significantly elevated over baseline[5]. When considering an appropriate dose in the cheetah, dose-response studies in domestic cats can be used as a guideline[5,3,13,14]. The recommended dose for intravenous tetracosactrin that results in maximal stimulation in domestic cats is reported to be 125 μg/cat, which translates into a dose of 25.0 μg/kg, with a range of 0.25 to 25 μg/kg[14]. The mean serum cortisol concentrations increased from 80.2 ± 5.0 nmol/ℓ to a peak of 786.3 ± 55.5 nmol/ℓ after a dose of 400 IU of slow release ACTH gel administered intramuscularly in 4 cheetahs[18].

The aim of this study was to determine the magnitude and timing of the serum cortisol response to the synthetic ACTH formulation available in South Africa in captive cheetah.

MATERIALS AND METHODS

Experimental animals

Sixteen cheetahs were used for this study. The cheetahs were of mixed origin; 6 were male and 10 were female. All animals were kept at Kapama Cheetah Research Station in Hoedspruit, South Africa. The 3 juveniles and 1 adult female were housed in the quarantine cages at the hospital and the remainder in bushveld enclosures grouped in pairs. Three juveniles (approximately 6 months of age) and 1 adult (approximately 2 years of age) were wild caught and housed in quarantine for approximately 14 days prior to the initiation of this study. The remaining cheetahs housed in camps were adults; many were assessed to be geriatric. The average estimated mass of the juveniles was 15 kg and the mass of the adults varied from 35 to 47 kg. Table 1 includes the age category, sex and housing of the 16 cheetahs.

Immobilisation

All immobilisations were undertaken before the heat of the day at approximately 06:30. All the cheetahs in the camps were tested on the same day, and those cheetahs housed in quarantine were tested on the subsequent day. Animals were given an induction dose of 5.5 mg/kg of tiletamine/zolazapam (Zoletil®, Virbac, Centurion) delivered by a low impact gas-powered darting system (Dan-inject International SA, Skukuza) Top-up doses of Zoletil were given IV in 30–50 mg doses at intervals, when required, to maintain anaesthesia for 4 h.

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Received: April 2007. Accepted: August 2007.

Table 1: **Age category, sex and housing of the cheetahs.**

Cheetah ID	Age category	Sex	Housing*
1	Adult	Male	BE
2	Adult	Male	BE
3	Adult	Female	BE
4	Adult	Male	BE
5	Adult	Female	BE
6	Adult	Male	BE
7	Adult	Female	BE
8	Juvenile	Female	QC
9	Juvenile	Male	QC
10	Juvenile	Male	QC
11	Adult	Female	QC
12	Adult	Female	BE
13	Adult	Female	BE
14	Adult	Female	BE
15	Adult	Female	BE
16	Adult	Female	BE

*BE, bushveld enclosure; QC quarantine cage.

The respiratory rate, heart rate, mucous membrane colour, capillary refill time, and body temperature, were monitored regularly and recorded hourly. Animals were placed in transport crates in the shade during the recovery phase.

Treatment and sampling

Eight cheetahs were randomly designated to the treatment group and 8 were designated as controls. Immediately after induction an 18-gauge intravenous catheter (Jelco, Johnson and Johnson, Midrand) was placed in the cephalic vein. A blood sample was collected as close to the time of induction as possible and again 30 min later to estimate serum cortisol concentrations prior to the administration of tetracosactide to the animals from the treatment group. All animals were placed on a constant intravenous infusion of Ringer-lactate solution® (Fresenius Kabi, Midrand). After the second blood sample was taken, 500 μg of synthetic adrenocorticotrophic hormone, tetracosactide (Synacthen Depot®, Novartis, Kempton Park), was administered by a slow intravenous injection. The control animals were given an identical volume of saline intravenously. Blood samples were collected in serum vacutainer tubes every 30 min for a total of 180 min thereafter. The blood samples were allowed to clot and then centrifuged at 1000 *g* for 10 min. The serum was collected and stored refrigerated in cryogenic tubes at the Research Station's hospital facilities at 1 °C, and then transported on ice to the laboratories at the Faculty of Veterinary Science, University of Pretoria, where the analysis was done.

Serum analysis

Serum cortisol concentration was determined using a radio-immunoassay validated for cat plasma (Clinical Assays Gamma Coat™Cortisol ^{125}I RIA, DiaSorin, Stillwater, Minnesota, USA).

Data analysis

Serum concentrations of cortisol were compared between the 2 groups for the time intervals specified. Data was not normally distributed and therefore median cortisol concentrations at corresponding time-points between the 2 groups were compared using the Mann-Whitney *U*-test. Within group differences in median cortisol concentrations were compared using Friedman's test, which is the non-parametric equivalent of the repeated measures ANOVA for related samples. Unless otherwise stated, cortisol is expressed as a median and inter-quartile range. Statistical analysis was performed on a personal computer using SPSS 14, 2005, SPSS Inc, Chicago, Illinois, USA. A *P*-value less than 0.05 was considered significant.

RESULTS

The individual results for the ACTH-stimulated and saline-treated cheetahs are shown in Tables 2 and 3, respectively. The 180-min. sample for cheetah nos. 1, 2 and 7 were not collected as these animals were gaining consciousness and it was decided that to top-up the anaesthetic at this late stage would be an unnecessary risk.

Baseline serum cortisol concentrations, before treatment, for both the control and ACTH-stimulated animals varied from 61.6 nmol/ℓ to as much as 250.3 nmol/ℓ among individual cheetahs. The median serum cortisol concentration at time 0 min for this group of 16 cheetahs was 120 (78–173) nmol/ℓ. The median cortisol concentration, as measured before the groups were subjected to their respective treatments, was 165.46 (82.23–225.63) nmol/ℓ and 119.11 (76.40–142.89) nmol/ℓ for the control and ACTH group, respectively. The results of the Friedman test indicated that there was no difference in the median cortisol concentration across all the time points in the control group ($P = 0.238$). There was a significant difference in the median cortisol concentrations of time points 30 to 180 min in the ACTH group after stimulation with ACTH ($P < 0.001$).

The cortisol responses for both the ACTH-stimulated and the control cheetahs are represented in Fig. 1. It depicts the cortisol concentrations in the ACTH-stimulated group, which increased signif-

Table 2: **Serum cortisol concentrations** (nmol/ℓ) **of cheetahs at time intervals of 30 min. The ACTH was administered at time period 0 min, thus baseline results occurred at time period –30 min and 0 min. No sample was collected for cheetah no. 2 at 180 min.**

Cortisol (nmol/ℓ)	–30 min	0 min	30 min	60 min	90 min	120 min	150 min	180 min
2	147.41	121.22	426.81	514.81	538.25	576.73	665.81	
4	217.64	150.11	543.18	612.81	890.90	948.20	940.21	926.91
6	225.20	206.36	445.95	533.05	576.10	566.11	697.79	796.40
8	85.96	71.06	320.49	454.32	475.89	534.40	619.86	507.43
10	148.64	118.88	446.03	471.54	525.96	576.88	664.73	531.56
12	98.11	92.45	433.53	452.39	540.74	648.82	633.39	630.03
14	89.77	61.56	429.10	592.58	419.72	768.01	755.15	768.52
15	199.76	119.34	632.60	706.08	857.95	823.05	966.61	1132.10
Median		119.11	439.74	523.93	539.50	612.85	681.80	768.52
25th percentile		76.41	427.74	523.93	539.50	612.85	681.80	768.52
75th percentile		142.89	518.90	607.75	787.49	809.29	893.95	926.91

Table 3: **Serum cortisol concentrations** (nmol/ℓ) **of cheetahs at 30 min intervals. Saline as a placebo control procedure was injected at time period 0 min. No samples were collected from cheetah nos. 1 and 7 at time period 180 min.**

Cortisol (nmol/ℓ)	**–30 min**	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**	**150 min**	**180 min**
1	205.83	250.26	173.54	148.59	122.77	137.89	109.43	
3	202.40	172.91	107.95	102.35	87.28	95.24	75.68	737.44
5	185.57	173.61	147.74	148.53	126.80	106.53	85.57	80.49
7	161.06	158.00	122.71	105.00	114.31	122.04	122.91	
9	106.02	73.32	65.68	104.10	65.80	98.97	242.72	144.67
11	114.34	65.69	72.69	42.54	57.82	35.97	38.21	84.34
13	188.14	108.97	84.60	900.84	45.20	35.97	41.52	66.50
16	334.07	242.97	412.62	697.85	634.17	401.75	255.01	207.97
Median		165.46	115.33	126.77	100.80	102.75	97.50	114.50
25th percentile		82.23	75.67	102.79	59.81	50.78	50.06	76.99
75th percentile		225.63	167.10	560.54	125.79	133.92	212.77	340.34

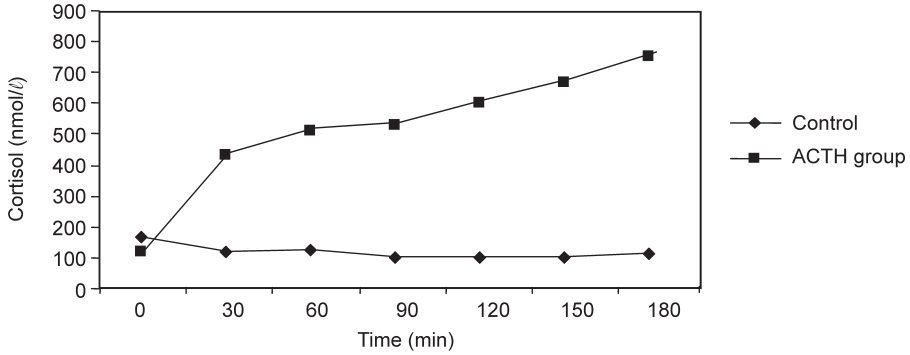

Fig. 1: **Median serum cortisol concentrations** (nmol/ℓ) **determined at time intervals of 30 min in ACTH-stimulated cheetahs and control cheetahs which were given saline.**

icantly by 30 min after the treatment and remained significantly raised to peak at 120 to 180 min following ACTH stimulation. This increase was absent in the control group that were given saline. There was a statistically significant ($P < 0.01$) difference in the cortisol concentrations of the ACTH-stimulated group at 4 of the 5 post-ACTH injection times when the results were compared to the saline controls at the equivalent times. The differences in the 60 min time point approached significance ($P = 0.063$). This lack of a significant difference resulted from unexplained cortisol peaks at the 60 min time point in 2 cheetahs from the control group.

Variation was seen in the time of peak cortisol response after ACTH. Two cheetahs peaked at 120 min, 3 at 150 min and 3 at 180 min after ACTH stimulation, but statistically significant differences in cortisol concentration between these 3 time points could not be demonstrated (Friedman's test, $P = 0.867$). The post-ACTH peak median serum cortisol concentration ranged from 612.85 (568.77–809.29) nmol/ℓ at 120 min and 681.8 (641.22–893.95) nmol/ℓ at 150 min to 768.52 (531.56–926.91) nmol/ℓ at 180 min.

DISCUSSION

The results from this study demonstrated a marked rise in serum cortisol concentrations after the intravenous administration of tetracosactide in all 8 cheetahs. In contrast, there was no significant cortisol increase in the serum of the 8 control cheetahs following the intravenous saline, despite them being subjected to considerable perceived stressors such as darting and general anaesthesia.

The median cortisol concentration, as measured before the groups were subjected to their respective treatments, was higher than the mean basal cortisol in domestic cats, which has been reported to be 33.0 nmol/ℓ[1], 40.6 nmol/ℓ[11], 32.1 nmol/ℓ[13] and, 79.8 nmol/ℓ[16] but more comparable to the mean basal concentrations found client-owned cats brought to the hospital on the morning of sampling, 203.0 nmol/ℓ[14]. Domestic cats subjected to the stress of handling and anaesthesia, were found to have a mean cortisol concentration of 193 nmol/ℓ[21]. The relatively high basal cortisol concentration in the cheetah may be a response to underlying captivity stress, darting or general anaesthesia or merely the stress of handling, as demonstrated by the 2 latter domestic cat studies[14,21].

The temporal response measured following the intravenous administration of ACTH in our study is consistent with tetracosactrin studies in the domestic cat[1,12,14,16]. After intravenous administration of tetracosactide, the cortisol began to rise within 30 min and remained elevated for at least a further 3 hours. The peak concentration occurred between 120 and 180 min. The 4 cheetahs that were given an intramuscular dose of 400 U of slow-acting ACTH gel, had similar cortisol responses over time to the animals in this study[18]. The cortisol concentration in those 4 cheetahs started to rise within 10 min, remained elevated for the entire duration of blood sampling (at least 120 min), and peaked from 40 to 120 min after the ACTH injection, with a mean peak value of 786.3 ± 55.5 nmol/ℓ[18]. When the adrenal responses of the cheetahs in our study are compared to those 4 cheetahs stimulated by ACTH gel intramuscularly, the synthetic depot ACTH formulation used by us produced an adrenal response later and for a longer time[18]. The results of a similar investigation using the same formulation of tetracosactide (Synacthen Depot®) given intravenously in domestic cats have been reported[1]. The highest cortisol concentration was measured at 90 min, the mean cortisol concentrations at 60, 90 and 120 min did not differ significantly, and the cortisol concentration returned to basal values by 180 min in all of the cats. The mean post-ACTH stimulation peak cortisol concentrations in the domestic cats (185 ± 32.9 nmol/ℓ in intact cats and 221.0 ± 97.3 nmol/ℓ in ovariohysterectomised animals), was much lower than that found in our group of cheetahs (612.85–768.52 nmol/ℓ). The results in our and those of the previous cheetah study were similar and clearly demonstrated that the peak serum cortisol concentrations attained by the cheetah is much higher than those seen in dogs and cats. Since the ACTH stimulation test is a test of adrenal reserve, we believe that this response in captive cheetahs demonstrates a species difference together with the effects of captivity. The results of the ACTH stimulation test in our study thus support the finding of adrenal cortical hypertrophy in captive cheetahs[9].

From the cortisol response curve it appears as though a limitation of our study was the fact that serial cortisol measurement was not continued for a sufficient period of time. Although the majority of cheetahs peaked between 120 and 180 min, it cannot be determined from our data whether cheetahs numbered 6, 14 and 15 in fact peaked at 180 min, or if their cortisol would have continued to rise.

Acknowledging the shortcomings of observing adverse effects under general anaesthesia, no serious adverse effects were noted following the intravenous administration of the depot formulation of ACTH in this study. Vomiting after the intravenous administration of tetracosactrin has been reported in cats[14,16]. The dose of 500 μg given to the treated cheetah in this study amounted to a dose range of 11.1 to 33.3 μg/kg, which broadly overlapped with the published domestic cat dose range of 0.25 to 25.0 μg/kg.

The necessity of time-related sampling over a prolonged period, as required by the ACTH stimulation test, militates against the procedure being undertaken in a wild animal that is not sedated or anaesthetised. The stress of manual restraint and repetitive blood sampling can be expected to cause a stress response in these animals. The influence of anaesthesia on cortisol secretion has been examined in cats. These studies indicate that some sedative or anaesthetic drugs do affect the animals' response to the administration of ACTH. Cats anaesthetised with etomidate were not capable of responding to ACTH, whereas cats anaesthetised with ketamine-diazepam responded with an appropriate increase in serum cortisol concentrations[5]. The results of this study demonstrate that the tiletamine/zolazapam combination did not affect the cheetahs' ability to respond to ACTH. In addition, the anaesthetic on its own did not cause any significant rise in serum cortisol concentrations throughout the 3 hours of anaesthesia.

The intravenous use of a depot formulation of synthetic ACTH in cheetah is a safe, effective method of assessing adrenocorticol function. At the dose of 500 μg per cheetah the cortisol concentrations should be expected to start rising after 30 min and peak anywhere between 120 to 180 min.

ACKNOWLEDGEMENTS

The authors would like to thank the South African Veterinary Foundation and the Faculty of Veterinary Science of the University of Pretoria for financial support. We also thank Mrs A Human at the Veterinary Theriogenology Department and Mrs M Mülders at the Department of Pharmacology, University of Pretoria for their technical support. Further, the research would not have been possible without the permission of Mrs Lente Roode and the invaluable assistance of Dr P Rogers and his team at The Hoedspruit Endangered Species Centre. This report emanates from project 36.5.466 approved by the Animal Use and Care and Research Committees, Faculty of Veterinary Science, University of Pretoria.

REFERENCES

1. Chatdarong K, Ponglowhapen S, Karlsson A, Forsberg C L 2006 The effect of ACTH stimulation on cortisol and progesterone concentrations in intact and ovariohysterectomized domestic cats. *Theriogenology* 66(6-7): 1482–1487
2. Deusberg C, Peterson M E 1997 Adrenal disorders in cats. *Veterinary Clinics of North America: Small Animal Practice* 27(2): 321–347
3. Essawy S A, Benhaj K M, Cooke R G, Dobson H 1989 Effect of dose and route of administration of $ACTH_{1-24}$ on plasma cortisol concentrations in ewes. *Journal of Veterinary Pharmacology* 12: 302–306
4. Feldman E C, Tyrrell J B 1977 Adrenocorticotropic effects of synthetic polypeptide-alpha 1-24 corticotropin in normal dogs. *Journal of the American Animal Hospital Association* 13: 494–499
5. Kemppainen R J, Mansfield P D, Sartin J L 1983 Endocrine responses of normal cats to TSH and synthetic ACTH administration. *Journal of American Animal Hospital Association* 20: 737–740
6. Lobetti R G, Picard J, Kriek N P J, Rogers P S 1998. Prevalence of helicobacteriosis and gastritis in a group of semi-captive cheetahs (Acinonyx *jubatus*) in South Africa. *Proceedings of a Symposium on Cheetahs as Game Ranch Animals, Onderstepoort, Pretoria,* 23–24 October 1998: 123–130
7. Moon P F 1997 Cortisol suppression in cats after induction of anaesthesia with etomidate, compared with ketamine-diazapam combination. *American Journal of Veterinary Research* 58(8): 868–871
8. Munson L 1993 Disease of captive cheetahs (*Acinonyx jubatus*): results of the cheetah research council pathology survey, 1989–1992. *Zoo Biology* 12: 105–124
9. Papendick R E, Munson L, O'Brien T D, Johnson K H 1997 Systemic AA amyloidosis in captive cheetah *(Acinonyx jubatus). Veterinary Pathology* 34: 549–556
10. Peterson M E, Kintzer P P, Foodman M S, Piccolie A, Quimby F W 1984 Adrenal function in the cat: comparison of the effects of cosyntropin (synthetic ACTH) and corticotropin gel stimulation. *Research in Veterinary Science* 37: 331–333
11. Peterson M E, Kemppainen R J 1992 Comparison of intravenous and intramuscular routes of administering cosyntropin for corticotropin stimulation in cats. *American Journal of Veterinary Research* 53(8): 1392–1395
12. Peterson M E, Kemppainen R J 1992 Comparison of the immunoreactive plasma corticotropin and cortisol responses to 2 synthetic corticotropin preparations (tetracosactrin and cosyntropin) in healthy cats. *American Journal of Veterinary Research* 53(8): 1752–1755
13. Peterson M E Kemppainen R J 1993 Dose-response relation between plasma concentrations of corticotropin and cortisol after administration of incremental doses of cosyntropin for corticotropin stimulation testing in cats. *American Journal of Veterinary Research* 54: 300–304
14. Schoeman J P, Evans H J, Childs D, Herrtage M E 2000 Cortisol responses to two different doses of intravenous synthetic ACTH (tetracosactrin) in overweight cats. *Journal of Small Animal Practice* 41: 552–557
15. Smith M C, Feldman E C 1987 Plasma endogenous ACTH concentrations and dexamethasone sodium phosphate in healthy cats. *American Journal of Veterinary Research* 48: 1719–1723
16. Sparks A H, Adams D T, Douthwaite J A, Gruffydd-Jones T J 1990 Assessment of adrenal function in cats: response to intravenous synthetic ACTH. *Journal of Small Animal Practice* 31: 2–5
17. Terio K A, Solnick J V, Munson L 1998 Potential mechanisms underlying gastritis in cheetahs (*Acinonyx jubatus*). *Proceedings of a Symposium on Cheetah as a Game Ranch Animal, Pretoria, South Africa,* 23–24 October 1998: 131–135
18. Terio K, Citino S B and Brown J L 1999 Fecal cortisol metabolite analysis for non-invasive monitoring of adrenocortical function in the cheetah (*Acinonyx jubatus*). *Journal of Zoo and Wildlife Medicine* 30(4):484–491
19. Terio K A, Marker L and Munson L 2004 Evidence for chronic stress in captive but not free ranging cheetahs (*Acinonyx jubatus*) based on adrenal morphology and function. *Journal of Wildlife Diseases* 40(2):259–266
20. Wack R F, Eaton K A, Kramer L W 1997 Treatment of gastritis in cheetahs *(Acinonyx jubatus*). *Journal of Zoo and Wildlife Medicine* 28: 260–266
21. Willemse T, Vroom M W, Mol J A, Rijnberk A 1993 Changes in plasma cortisol, corticotropin, and α-melanocyte-stimulating hormone concentrations in cats before and after physical restraint and intradermal testing. *American Journal of Veterinary Research* 54(1):69–71