COMPARATIVE RESPONSE OF BLOOD CORTISOL LEVELS TO TWO DIFFERENT DOSES OF INTRAVENOUS ACTH (TETRACOSACTRIN) IN OVERWEIGHT CATS

by

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SUMMARY

Fifteen middle-aged to older, overweight cats were investigated to rule out hyperadrenocorticism as a cause of their weight problem, using two different protocols for the adrenocorticotrophic hormone (ACTH) stimulation test. The cats received intravenous synthetic ACTH (tetracosactrin) at a dose of 125 µg initially and then, between 2 and 3 weeks later, a second test was performed using a dose of 250 µg intravenously. The peak of the mean serum cortisol concentrations taken at all time points, occurred at 60 minutes following the 125 µg dose and at 120 minutes following the 250 µg dose. There was no statistically significant difference between the cortisol peaks attained using either dose of tetracosactrin. There was, however, a significantly higher serum cortisol concentration attained after the higher dose at the 180 minutes time point, indicating a more prolonged response, when compared with the lower dose.

The mean basal cortisol concentration was 203 nmol/l (range 81 – 354 nmol/l).

The cats were followed up for one year after the initial investigations. Urine obtained one year later in the cats' home environment, showed a mean urinary cortisol/creatinine ratio (UCCR) of 3.3×10^{-6} (range $0.85 - 8.67 \times 10^{-6}$). A mean weight loss of 6 per cent was achieved over the period of the study. The weight loss, lack of development of clinical signs and the normal UCCR's confirm that none of these cats had gone on to develop hyperadrenocorticism.

SAMEVATTING

‘n Kliniek populasie van vyftien middeljarige tot ouer katte is ondersoek om hiperadrenokortikisme uit te skakel as die oorsaak van hul oorgewig-probleem. Dit is gedoen deur gebruik te maak van twee protokolle vir die adrenokortikotrofiese hormoon stimulasie toets.

Die katte is aanvanklik ingespuit met ‘n dosis van 125 µg intraveneuse adrenokortikotrofiese hormoon (ACTH) en bloed monsters is geneem voor en op 60, 120 en 180 minute na die inspuiting. Hierdie toets is opgevolg met ‘n tweede ACTH-stimulasie toets, twee tot drie weke later, wat gebruik gemaak het van die hoër dosis van 250 µg.

Die gemiddelde basale bloed kortisol konsentrasie was 203 nmol/l (reikwydte 81 – 354 nmol/l).

Die hoogste gemiddelde serum kortisol konsentrasie het voorgekom op 60 minute na die laer dosis, maar op 120 minute na die hoër dosis. Daar was egter geen statistiese beduidende verskil tussen hierdie twee kortisol konsentrasies nie.

‘n Beduidend hoër kortisol konsentrasie is egter behaal op die 180 minuut tydstip na die hoër dosis van 250 µg, wat dui op ‘n meer volgehoue respons na die toediening van die hoër dosis, in vergeleke met die laer dosis.

Hierdie katte is opgevolg vir een jaar na die ACTH stimulasie toetse om te bepaal of enige van hulle hiperadtrenokortikisme sou ontwikkel. Urienmonsters is een jaar na die ACTH-stimulasie toetse in die katte se tuis omgewing gekollekteer en urien kortisol/kreatinien verhoudings is bepaal. Die resultate hiervan was binne normale perke en tesame met die matige gewigsverlies, het dit op die afwesigheid van hiperadrenokortikisme gedui.

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LIST OF ABBREVIATIONS

ACTH	=	Adrenocorticotrophic hormone
ADH	=	Adrenal dependent hyperadrenocorticism
BCS	=	Body condition score
DSH	=	Domestic shorthair
^{0}C	=	degrees Celsius
HAC	=	Hyperadrenocorticism
HPA axis	=	Hypothalamic-pituitary-adrenal axis
IU/kg	=	International units per kilogram
mg/kg	=	milligram per kilogram
nmol/l	=	nano mol per litre
%	=	percent
PDH	=	Pituitary dependent hyperadrenocorticism
PU/PD	=	Polyuria and Polydipsia
RIA	=	Radio-immunoassay
RPM	=	Revolutions per minute
UCCR	=	Urine cortisol: creatinine ratio
µg	=	microgram
µg/kg	=	microgram per kilogram
µmol/l	=	micromol per litre
2SD	=	two standard deviations

1. LITERATURE REVIEW

1.1 FELINE HYPERADRENOCORTICISM

1.1.1 Definition

Hyperadrenocorticism (HAC) can occur spontaneously as a disorder resulting from the excessive production of glucocorticoids by the adrenal cortex. As in the dog, hyperadrenocorticism in the cat can arise from disease of either the pituitary or adrenal gland. An iatrogenic form exists, that can occur as a result of the long-term administration of exogenous glucocorticoids, although cats seem to be particularly resistant to the effects of glucocorticoids (Scott 1982).

1.1.2 Pathophysiology of hyperadrenocorticism

Pituitary-dependant hyperadrenocorticism (PDH) results from excessive secretion of adrenocorticotrophic hormone (ACTH) from the pars distalis or pars intermedia of the pituitary gland, which in turn induces bilateral adrenocortical hyperplasia and hypercortisolaemia (Nelson and others 1988). Excess secretion of ACTH may arise from neoplastic or, less commonly, hyperplastic pituitary corticotroph cells. Adrenal-dependent hyperadrenocorticism (ADH) result from adrenocortical tumours that secrete excessive cortisol autonomously, escaping the normal regulatory influence of ACTH from the pituitary gland. An equal distribution of benign and malignant adrenal tumours exists in both the cat and the dog (Duesberg and Peterson 1997). As in the dog, approximately 80% of cases of feline hyperadrenocorticism result from pituitary disease and the remaining 20% from adrenal disease.

1.1.3 Previously published cases

In cats HAC is a rare endocrine disorder and was first recognised and published as a single case report by Fox and Beatty (1975). This case presented as a complicated case of diabetes mellitus. The surgical treatment of one other case was described soon after (Swift and Brown 1976) and was followed by the description of the disease due to an adrenocortical adenoma in a cat (Meijer and others 1978).

It was not until 1986, when a few more cases were recognised, that a renewed interest in feline adrenocortical disorders took place. Since 1986, several individual case reports (Peterson and Steele 1986, Zerbe and others 1987, Usher 1991, Jones and others 1992, Furuzawa and other 1992, Kipperman and others 1992, Daley and others 1993, Valentine and Silber 1996, Schwedes 1997) and multi-case reviews (Nelson and others 1988, Peterson 1988, Zerbe 1989, Immink and others 1992, Van Sluijs and Sjollema 1992, Duesberg and others 1995, Watson and Herrtage 1998, Deusberg and Peterson 1997) have greatly expanded the literature on spontaneous HAC in cats.

There are, however, currently only about 70 cases of feline HAC reported in the veterinary literature (Mooney 1998). Over 80 % of feline HAC cases in the literature presented with concurrent diabetes mellitus, which is considered to be a late complication of the disease in dogs (Duesberg and Peterson 1997).

1.1.4 Clinical signs

The clinical signs, clinicopathological and radiological findings in feline HAC are generally less dramatic than in dogs, which might explain the reason why feline cases are diagnosed in a relatively later stage of the disease. Weight gain and a potbellied

appearance are reported to be amongst the most frequently recognised signs of HAC in cats (Nelson and others 1988, Myers and Bruyette 1994, Duesberg and Peterson 1997). Other clinical signs include polyuria, polydipsia (PU/PD), polyphagia, generalised muscle wasting and lethargy. Many of the reported cases had dermatological abnormalities including thin, fragile skin, truncal alopecia and recurrent infections. Less frequently noted signs include hepatomegaly and an unkempt haircoat (Zerbe 1989).

Polyuria and polydipsia in the dog with HAC is believed to occur as a result of the inhibition of either the action or secretion of ADH by the circulating glucocorticoids. In contrast, the onset of polyuria and polydipsia in the cat with HAC coincides with the development of osmotic diuresis secondary to severe hyperglycaemia and associated glucosuria (Scott and others 1982, Zerbe 1989).

In the latest retrospective study of six cases of feline HAC, all cases were described as overweight, but more importantly, three of these cases presented without diabetes mellitus (Watson and Herrtage 1998). The mechanism of diabetes mellitus secondary to HAC, is believed to be due to insulin resistance, mediated directly by a reduction in number and efficacy of glucose transporters and indirectly by increasing the circulating levels of glucagon and free fatty acids (Moller and Flier 1991). HAC cases without diabetes mellitus can reasonably be assumed to be in an earlier stage of the disease. As illustrated in the study by Watson and Herrtage (1998), heightened awareness of the disease probably results in earlier recognition before the onset of diabetes mellitus.

1.1.5 Clinicopathological findings

A marked degree of hyperglycaemia, exceeding the renal threshold, is the most common laboratory abnormality found on the serum biochemistries of the previously reported cases (Duesberg and Peterson 1997). Cats seem more sensitive to the diabetogenic effects of glucocorticoid excess than dogs (Peterson 1988, Zerbe 1989). Hypercholesterolaemia is also common and may relate to insulin resistance and increased lipolysis. Cats lack the steroid-induced isoenzyme of alkaline phosphatase found in the dog, and the half-life of the enzyme seems to be significantly shorter in the cat (Zerbe 1989, Nelson 1991). Other biochemical and haematological changes are non- specific and often reflect concurrent diseases. In contrast to the dog, cats with HAC do not consistently develop the characteristic clinicopathological abnormalities, so typical of the disease in the dog. The lack of the steroid-induced isoenzyme and the late onset of PU/PD means that two of the most consistent and characteristic signs in the dog are absent in the cat (Duesberg and Peterson 1997).

1.2 Diagnosis of feline hyperadrenocorticism

A great deal of information exists regarding the normal hypothalamic-pituitary-adrenal (HPA) axis in dogs, and the diagnostic approach to both adrenocortical hyperfunction and hypofunction is well established. In the cat, work in this field is limited and results contradictory. Work on the HPA axis in the cat has relied on evaluation of the ACTH stimulation test (Johnston and Mather 1979, Kemppainen and others 1984, Peterson and others 1984, Smith and Feldman 1987, Watson and others 1989, Sparkes and others 1990, Peterson and Kemppainen 1992a, Peterson and

Kemppainen 1992b, Peterson and Kemppainen 1993, Moon 1997), the high and low dose dexamethasone suppression tests (Medleau and others 1987, Smith and Feldman 1987, Peterson and Graves 1988) and the urine cortisol: creatinine ratio (Goossens and others 1995, Henry and others 1996). The ACTH stimulation test is currently the preferred test for screening for feline HAC.

1.2.1 Low dose dexamethasone suppression test

When evaluating the cortisol response to dexamethasone in cats, it becomes very clear that cats do not respond as predictably as dogs do. Normal cats are much more inconsistent with respect to the degree and duration of adrenocortical suppression following dexamethasone administration. A number of different protocols and sampling intervals have been evaluated (Medleau and others 1987, Peterson and Graves 1988, Smith and Feldman 1987). Several investigators have noted non-suppression in stressed or ill cats, even though no other evidence for hyperadrenocorticism was felt to exist (Zerbe and others 1987, Peterson 1988). Unfortunately, a large number of cats with confirmed hyperadrenocorticism have not been evaluated systematically to draw any firm conclusions as to which protocol is the most accurate in arriving at a correct diagnosis.

1.2.2 Urine cortisol to creatinine ratio

The value of the assessment of urinary corticoids in the diagnosis of canine hyperadrenocorticism was first demonstrated by Stolp and others (1983). Determination of a spot urine cortisol to creatinine ratio (UCCR) has been shown to

be an excellent screening test for HAC in dogs (Smiley and Peterson 1993). Work by Henry and others (1996) have shown that the mean UCCR for healthy cats was 5.9×10^{-6}(range $0.6 – 27 \times 10^{-6}$). Goossens and others (1995) have found no overlap in the range of UCCR in healthy cats when compared with cats with HAC and concluded, as is the case with the dog, that the utility of the test lies in excluding hyperadrenocorticism in a cat with a normal UCCR, ie. the test has a high negative predictive value. The test therefore appears to be a very sensitive test for feline HAC, although unfortunately lacking considerably in specificity. Rochlitz (1997) used the same methodology as was used in this study and found the mean UCCR in cats to be 5×10^{-6} (range $2 – 8.4 \times 10^{-6}$).

1.2.3 Adrenocorticotrophic hormone stimulation test

The ACTH stimulation test is a widely accepted diagnostic screening test for adrenocortical abnormalities in both humans and animals (Bruyette 1994).

The protocol for ACTH stimulation testing in dogs recommends a dose of 125 µg of tetracosactrin intravenously for dogs less than 5 kg and 250 µg for dogs over 5 kg (Herrtage 1998).

1.2.3.1 Principle of the ACTH stimulation test

The rationale behind the ACTH stimulation test relies on cortisol secretion by the adrenal glands after being stimulated by a supraphysiological dose of exogenous ACTH. Basal serum or plasma cortisol concentrations are obtained prior to the injection of ACTH and at varying time intervals after the injection. Cats with enlarged

adrenal glands due to either chronic hypophyseal ACTH stimulation or adrenal neoplasia should hyper-respond, whilst cats with atrophied or destroyed adrenal glands should show a lack of response. False negative results can occur if the adrenal glands show a lack of response to ACTH due to an autonomous cortisol secreting tumour, whilst false positive tests can occur in chronically stressed animals or those with other diseases like hyperthyroidism and diabetes mellitus (Zerbe and others 1987).

1.2.3.2 Review of research on the ACTH stimulation test in cats

Various authors have evaluated the ACTH stimulation test in laboratory cats. These studies used a variety of ACTH preparations at different doses, routes of administration and sampling times.

The first study to document the cortisol response to ACTH in cats was by Johnston and Mather (1979). They found a mean basal cortisol concentration of 47 nmol/l and demonstrated detectable increases in plasma cortisol concentrations 1 to 2 hours after the intramuscular injection of 2.2 U of ACTH gel. Only six cats were used in this study and whilst they all reponded to the dose, they all peaked at different times after the ACTH and firm conclusions could thus not be drawn.

Kemppainen and others (1984) injected doses of 125 μg and 1μg/kg to two groups of 4 cats respectively. They found that the intramuscular injection of 1μg/kg provided very weak stimulation and produced serum cortisol values similar to those in the saline controls. They did, however, demonstrate a marked response to the 125ug dose and suggested that the peak serum cortisol concentration occurred at 30 minutes

post intramuscular injection. All the cats in this group showed a rapid decline in their serum cortisol levels, reaching near normal values after 90 minutes.

Peterson and others (1984) compared the responses obtained using 125 µg of intravenous cosyntropin on the one hand and 2.2 IU of intramuscular ACTH gel on the other. They found, and therefore recommended, that sampling should be done at 60 – 90 minutes following intravenous injection with cosyntropin and at 90 – 120 minutes following injection with ACTH gel. Curiously, maximal responses were higher following the intramuscular injection of the gel, than following intravenous injection with cosyntropin, demonstrating that higher cortisol concentrations can be obtained than those obtained after 125 µg of intravenous cosyntropin.

Smith and Feldman (1987) compared the results of the intramuscular ACTH stimulation test using synthetic ACTH (cosyntropin) at a dose of 125 µg in five cats, with a dose of 250 µg in six different cats and found no significant difference between responses resulting from the two dosages. They concurred with the findings of Kemppainen and others (1984), in observing peaks at 30 minutes after intramuscular injection. All the cats weighed between 3.2 and 5.5 kg. Two of the cats receiving the 250 µg dose vomited and remained depressed for two hours. They concluded that the adverse reaction observed, should serve as a deterrent to the use of the 250 µg dose in cats.

The first report on the response of cats to intravenous synthetic ACTH (tetracosactrin), the preparation used in Europe, was by Sparkes and others (1990). They used a dose of 125 µg in fifteen barrier-maintained cats, weighing between 2.6 and 4.7 kg. They concluded that most of the cats produced peak serum cortisol concentrations at 180 minutes after tetracosactrin. Vomiting was noted as side effect in three of these cats.

Peterson and Kemppainen (1992a) compared the intramuscular and intravenous routes of administration, using a dose of 125 µg of cosyntropin in ten colony cats, weighing between 2.7 and 5 kg. They found that the intravenous route induced significantly greater and more prolonged adrenocortical stimulation than the intramuscular route. They also demonstrated a peak serum cortisol response between 60 and 90 minutes after intravenously administered cosyntropin, which was earlier than the peak reported by Sparkes and others (1990).

Presumably because of the discrepancy between the above-mentioned two studies, Peterson and Kemppainen (1992b) then evaluated the cortisol responses to the two available synthetic ACTH preparations (tetracosactrin and cosyntropin). They administered the preparations intravenously in ten cats and found that these preparations produced a comparable, if not identical pattern of response. In this study they noted that four cats which responded late to one preparation, also responded late to the other. This led to a change in their recommendation of sampling times after intravenous administration of ACTH, to include, not only a 60 to 90 minute sample, but also a sample at 120 to 180 minutes, to ensure that the peak response was not missed.

Peterson and Kemppainen (1993) then evaluated the serum cortisol responses after the intravenous administration of incremental doses of cosyntropin (1.25 µg, 12.5 µg and 125 µg) to six normal barrier-maintained cats weighing between 3 and 5 kg. They used indwelling jugular catheters for blood sampling and demonstrated comparable peak concentrations of serum cortisol at all three doses, but a more prolonged stimulation using the highest dose.

Crager and others (1994) administered synthetic ACTH (cosyntropin) at 125 µg intravenously to eight healthy cats ranging from 2.4 to 5.3 kg and showed that all cats had a higher serum cortisol concentration at 90 minutes than at 60 minutes.

1.2.3.3 Summary of research on the test

Following a study of the literature, it becomes clear that the reason for the confusion around the protocol for the ACTH stimulation test in cats, lies in the use of different ACTH preparations and routes of administration.

Intramuscular cosyntropin clearly produces the earliest peak in serum cortisol. Intravenous cosyntropin produces a higher peak than the intramuscular route and also showed a more prolonged response. The intramuscular gel produced a higher peak and even more prolonged response than the intravenous route. The gel is not commercially available any more.

In the six studies in which synthetic ACTH (cosyntropin or tetracosactrin) was administered intravenously in healthy cats, 125 µg was the highest dose used. All these cats weighed less than 5.3 kg and no mention were made of their respective body condition scores (Peterson and others 1984, Sparkes and others 1990, Peterson and Kemppainen 1992a, Peterson and Kemppainen 1992b, Peterson and Kemppainen 1993, Crager and others 1994).

2. PROBLEM

1. All previous studies of intravenous ACTH stimulation testing in cats have been carried out in young, laboratory conditioned cats, which may not truly represent the responses seen in a clinic-based population.

2. Sampling in these studies has mostly been performed from pre-placed jugular catheters after periods of acclimatisation. This is expected to have reduced the basal serum cortisol concentrations, influencing the interpretation of the degree of subsequent stimulation.

3. Some authors have also demonstrated increased hypothalamic-pituitary-adrenal activity in older animals, casting doubt on the validity of data obtained in young cats. Goossens and others (1995) demonstrated increased urinary excretion of glucocorticoids with increasing age in cats. This is also true for dogs, where increased activity of the hypothalamic-pituitary-adrenal axis was demonstrated in older dogs, resulting in higher plasma concentrations of ACTH and cortisol and higher urine cortisol/creatinine ratios (Rothuizen and others 1993).

4. The mean bodyweight of the cats used in previous ACTH stimulation test studies is significantly lower than the bodyweight of cats suspected of having hyperadrenocorticism.

5. Furthermore, false negative results are reported to have been obtained with the ACTH stimulation test in 33 per cent of cats with hyperadrenocorticism (Duesberg and Peterson 1997). In almost all of these cases the ACTH stimulation test was performed using ACTH gel intramuscularly at 2.2 IU/kg or synthetic ACTH at 125 µg intramuscularly. The use of the lower dose of ACTH (and thus the sub-optimal stimulation) could have been responsible for the high percentage of false negative results cited in the literature.

   The use of higher doses of synthetic ACTH by the more reliable intravenous route might help to reduce the percentage of false negative results.

3. OBJECTIVES OF THIS STUDY

The aim of this study is to assess:

1. The blood cortisol responses to intravenously administered ACTH (tetracosactrin) in a middle-aged to older, clinic-based population of cats.

2. To ascertain whether any higher peak serum cortisol concentrations can be obtained by using the 250 µg dose rather than the 125 µg dose of intravenous ACTH (tetracosactrin) in cats over 5 kg.

4. BENEFITS OF THIS STUDY

As considerable confusion prevails regarding the optimal protocol for the ACTH stimulation test in felines, the intention of this study is to make a contribution to clarify the uncertainty currently existing around the testing of cats for HAC in the clinic situation.

5. MATERIALS AND METHODS:

5.1 EXPERIMENTAL MODEL/DESIGN:

This is an analytical, clinical study in which the cortisol responses obtained, is attributed to the exposure to an intravenous dose of tetracosactrin (ACTH).

5.2 STUDY LIMITATIONS:

Due to ethical constraints, the first "exposure" using the lower dose had to precede the second one and therefore a cross over design of the study was not possible. However, to minimize confounding, a "wash out period" of at least two weeks was observed between the two doses of tetracosactrin. Another possible limitation lay in declaring that these cats were normal and not suffering from hyperadrenocorticism at the time of the study. I endeavoured to circumvent that problem by following them up for twelve months and then obtaining urine for urine cortisol: creatinine ratios. This test has been shown to have a high negative predictive value for hyperadrenocorticism in cats, ie. to have a low incidence of false-negative results (Goossens and others 1995).

5.3 SAMPLING SIZE:

Fifteen healthy cats of both sexes, weighing more than 5 kg, were prospectively engaged.

5.4 SELECTION OF CASES:

The cats were identified from the records of the obesity clinic run at The Veterinary Hospital, Bishop's Stortford, Hertfordshire, England. The cats that had failed to lose weight, despite appropriate dietary advice given to their owners by the obesity clinic staff, were recruited. They were investigated to exclude early hyperadrenocorticism as a cause of their lack of weight loss.

Other abnormalities, such as endocrine disorders, organ failure and cardio-respiratory diseases were excluded, based on the results of a full clinical examination, thoracic auscultation and a haematology- and biochemistry screen. The haematology and biochemistry was performed on the QBC analyser and Vettest (Idexx Laboratories) respectively. Haematology included haematocrit, haemoglobin, mean cell haemoglobin concentration, total white blood cell count, granulocyte count, lymphocyte/monocyte count and platelet count. Biochemistry included the following parameters: total protein, albumin, globulin, alkaline phophatase, alanine aminotransferase, amylase, urea, creatinine, calcium, cholesterol, glucose, phosphate and total bilirubin. The clinical case histories were carefully scrutinised to ensure that none of the cats received any medication, apart from anthelmintics and flea control, in the six months preceding the study.

Each of these cats was awarded a body condition score based on a 9 point score system described by Laflamme (1997) and only included in the study if considered overweight (body condition score of 6 or greater).

Exclusion criteria:

Cats were excluded from the study if they:

1. Weighed less than 5 kg.
2. Had lost weight following dietary advice given to their owners.
3. Had received any drugs apart from flea products or anthelmintics in the six months prior to the study.
4. Had a body condition score of less than 6 out of 9.
5. Had abnormal cortisol responses in the hyperadrenocorticism range to the first (125 µg dose) of ACTH.
6. Failed to be presented for their second test between 2 to 3 weeks after their first test.
7. Showed any abnormalities on their clinical, haematological or biochemical examinations.
8. Had an abnormal urinary cortisol to creatinine ratio after a twelve-month follow up period.

5.5 BODY CONDITION SCORING

Due to the difference in frame size, regarding cats as overweight could not be based solely on bodyweight. A body condition scoring system based on one described in the literature by Laflamme (1997) was used. This body condition scoring system is simple in design, requiring only an intellectual addition to a routine physical examination. The system is based on a nine-point scoring system and details are as follows:

Score 1: **EMACIATED**. Ribs visible on shorthaired cats; no palpable fat pad; severe abdominal tuck; lumbar vertebrae and wing of ilia easily palpated.

Score 2: **VERY THIN**. Shared characteristics of score 1 and 3.

Score 3: **THIN**. Ribs easily palpable with minimal fat covering; lumbar vertebrae obvious; obvious waist behind the ribs; minimal abdominal fat.

Score 4: **UNDERWEIGHT**. Shared characteristics of score 3 and 5.

Score 5. **IDEAL**. Well proportioned; observe waist behind the ribs; ribs palpable with slight fat covering; abdominal fat pad minimal.

Score 6: **OVERWEIGHT**. Shared characteristics of score 5 and 7.

Score 7: **HEAVY**. Ribs not easily palpated with moderate fat covering; waist poorly discernible; obvious rounding of the abdomen; moderate abdominal fat pad.

Score 8: **OBESE**. Shared characteristics of score 7 and 9.

Score 9: **GROSSLY OBESE**. Ribs not palpable under heavy fat cover; heavy fat deposits over lumbar area, face and limbs; distention of abdomen with no waist; extensive abdominal fat deposits.

Figure 1: Photograph of a typical cat used in this study. Note the generalised obesity. Body condition score: 8

5.6 EXPERIMENTAL PROCEDURE:

ACTH stimulation test procedures were started between 9 and 10 am. On the morning of the first test, each cat was given 125 µg of synthetic ACTH (tetracosactrin) intravenously using either of the cephalic veins. Blood samples were obtained by needle venipuncture from the jugular vein immediately before and at 60, 120 and 180 minutes after injection of tetracosactrin. The result of the first test was analysed and a second ACTH stimulation test performed only if the result of the first test was within currently accepted normal limits (Sparkes and others 1990). After an interval of at least two weeks (range 2 - 3 weeks) a second ACTH stimulation test was performed using a dose of 250µg of tetracosactrin intravenously. Blood samples were again collected by repeated jugular venipuncture at the same time intervals.

The samples were collected into plain glass tubes and centrifuged at 5000 rpm for 5 minutes, within two hours of collection. The serum was harvested and stored at -18 ^{o}C until assayed. Serum cortisol concentrations were measured by radio-immunoassay (Corti-Cote, Cortisol Solid Phase Component System, Becton Dickinson UK Ltd, Oxford, UK). The inter-assay variation across the range 54 - 927 nmol/l was calculated to be 5.8 - 7.8 per cent. The intra-assay variation across the range 97 - 1040 nmol/l was calculated to be 4.7 - 7.9 per cent. The lower limit of quantification was calculated to be 20 nmol/l.

The cats were examined regularly at the obesity clinic for a twelve-month period after the ACTH stimulation tests. In order to exclude hyperadrenocorticism further, urine samples were obtained and urinary cortisol/creatinine ratios (UCCR's) determined from the cats still in the study, 12 months after the ACTH stimulation tests. Urine was obtained from 10 of the cats by cystocentesis in their home environment and from the

remaining 5 cats by free-flow samples obtained from the litter tray in the home, using non-absorbent aquarium gravel (Aquagravel; Madingley Mulch) as litter. Urine was collected in plastic syringes or into plastic serum blood tubes and frozen within 24 hours at -18 ^{0}C until assayed. Urinary cortisol was measured using a radio-immuno assay (RIA) test kit (Corti-Cote, Cortisol Solid Phase Component System, Becton Dickinson UK Ltd, Oxford, UK). Urinary creatinine concentrations were determined by spectrophotometry (Jaffe reaction) on an autoanalyzer (Beckman CX5CE). The urinary cortisol intra-assay variation across the range of 97 - 1040 nmol/l was 4.7 - 7.9 per cent and the inter-assay variation across the range of 91 - 1090 nmol/l was 6.6 - 7.4 per cent. The urinary creatinine had an intra- and inter-assay coefficient of variation of less than 0.1 per cent across the range 8206 - 33951 µmol/l.

5.7 DATA ANALYSIS:

Between dose comparisons were made with respect to serum cortisol concentrations obtained before, at 60, 120 and 180 minutes after the two doses of intravenous synthetic ACTH. All the intra-procedure cortisol concentrations were also compared to each other. The percentages of cats that peaked at varying times after the administration of each dose of tetracosactrin were determined. The mean peak serum cortisol concentration obtained after each dose was determined and compared to the other dose. The serum cortisol responses of each cat was also assessed individually. Statistical analysis of the serum cortisol concentrations obtained was performed as follows: normally distributed data was tested using the paired Student's t-test, otherwise the Wilcoxon Signed rank test was used. A p value of 0.05 or less was considered significant.

5.8 PROJECT MANAGEMENT:

5.8.1 EXPERIMENTAL ANIMALS

All the cats were client-owned and brought to the veterinary hospital on the morning of each test procedure, in an attempt to simulate the clinical situation in which this testing is normally done. Fully informed consent was obtained from the owners of the cats for obtaining the blood samples and performing the ACTH stimulation test, as well as for the cystocentesis procedure after the follow up period.

5.8.2 STAFF, FACILITIES AND EQUIPMENT

The author injected the ACTH and obtained all the blood samples and all the cats were manually restrained by the same experienced nurse. The facilities of the Veterinary Hospital in Bishop's Stortford, Hertfordshire, England were used and all materials, including blood tubes, needles and syringes, were supplied by the Veterinary Hospital. The study was conducted under the supervision of Dr. Michael Herrtage BVSc, MA, DVR, DVD, DSAM, DECVIM, DECVDI, MRCVS, Head of Small Animal Medicine and Radiology at Cambridge University Veterinary School, United Kingdom.

6. RESULTS

6.1 SIGNALMENT

The mean age of the cats was 8 years (range 5 - 11 years). The 15 cats consisted of six neutered females and nine neutered males (Table 1).

6.2 BODYWEIGHT

The mean weight of the 15 cats was 6.8 kg (range 5.1 - 9 kg) (Table 1).

The cats were followed up with regular weight checks and re-enforcement of the dietary program over the following year. A mean reduction of 6 per cent (range 0 - 14 per cent) in bodyweight was achieved during this period.

6.3 BODY CONDITION SCORING

Only cats with body condition scores of 6 and greater participated in the study. The mean body condition score of the cats was 7.7 (range 6 - 9) (Table 1).

6.4 CLINICAL EXAMINATION AND ASSESMENT

All haematology and biochemistry parameters were within the normal range. Clinical examinations revealed no abnormalities apart from obesity.

Table 1: Signalment, bodyweight and body condition scores of the 15 cats used in this study.

	AGE (YEARS)	SEX	BREED	BODYWEIGHT (KG)	BCS (6-9)
Cat 1	9.5	Female	DSH	7.4	8
Cat 2	9.5	Female	DSH	7.6	8
Cat 3	10	Male	DSH	7.8	9
Cat 4	6	Male	DSH	7.2	8
Cat 5	8	Male	DSH	6.8	8
Cat 6	11.5	Female	DSH	6.2	7.5
Cat 7	10	Female	DSH	5.5	7
Cat 8	9	Female	DSH	5.3	7
Cat 9	5	Male	DSH	5.1	7
Cat 10	6	Male	DSH	5.2	6
Cat 11	10.5	Male	DSH	9.0	8.5
Cat 12	5	Female	DSH	8.5	8
Cat 13	6	Male	DSH	7.7	9
Cat 14	11.5	Male	DSH	6.2	7
Cat 15	5	Male	DSH	6.5	7

6.5 SERUM CORTISOL RESPONSES

The results of the serum cortisol concentrations of individual cats are shown in Table 2 and in Figures 2 and 3. The basal serum cortisol concentrations for both ACTH stimulation test procedures (n=30) ranged from 81 to 354 nmol/l (mean 203 nmol/l). The mean basal serum cortisol concentration was 216 nmol/l at the start of the first procedure and 189 nmol/l before the second procedure. This difference was not significant ($p = 0.14$).

Mean serum cortisol concentrations increased following the administration of either dose of tetracosactrin. The time to reach maximal serum cortisol concentration was variable and tended to occur earlier following the lower dose. Following the 125 µg dose 6 out of 15 cats (40 %) peaked at 60 minutes, 6/15 cats (40 %) peaked at 120 minutes and 3 out of 15 (20 %) peaked at 180 minutes. Following the 250 µg dose 2 out of 15 cats (13 %) peaked at 60 minutes, 8 out of 15 cats (53 %) peaked at 120 minutes and 5 out of 15 cats (33 %) peaked at 180 minutes.

All cats had a marked response to tetracosactrin at 60 minutes. The two doses of tetracosactrin produced statistically similar results at both 60 and 120 minutes. The mean serum cortisol concentration at 60 minutes for the 125 and 250 µg doses were 308 and 288 nmol/l, respectively and at 120 minutes the concentration was 304 nmol/l for the 125 µg dose and 314 nmol/l for the 250 µg dose (Figure 4). The mean of the peak serum cortisol concentration at any time point in the 15 cats was 331 nmol/l (range 199 to 545 nmol/l) following the 125 µg dose and 321 nmol/l (range 173 to 514 nmol/l) following the 250 µg dose. The mean of the peak serum cortisol concentration achieved by the individual cats irrespective of the dose used, was 341 nmol/l (range 222 to 545 nmol/l).

However, analysis of the individual cortisol responses showed that two cats (cat 3 and 8) had a marked reduction in their serum cortisol concentrations between 60 and 120 minutes following the 125 µg dose, which was not as apparent following the 250 µg dose (Figures 2 and 3). The mean serum cortisol concentration at 180 minutes was significantly higher following the 250 µg dose (258 nmol/l) than it was following the 125 µg dose (200 nmol/l) ($p = 0.013$) (Figure 4). A more prolonged response thus occurred following stimulation with the 250 µg dose of tetracosactrin.

Nine out of 15 cats (60 %) peaked at similar times during both procedures. The three cats that peaked at 180 minutes following the 125 µg dose, also peaked at 180 minutes following the 250 µg dose (cat 6, 7 and 12). This would indicate that they were consistent late responders. The five cats that peaked at 180 minutes following the 250 µg dose, had peak values that did not differ significantly from their values at 120 minutes, indicating that stimulation had reached a plateau (cat 7, 8, 10, 12, 15).

The percentage increase in serum cortisol over baseline values ranged from 12 to 217 % (mean 63 %) following the 125 µg dose and from 16 to 200 % (mean 78 %) following the 250 µg dose.

One cat showed transient vomiting (cat 3). This occurred within minutes following the intravenous administration of both the 125 µg and 250 µg doses of tetracosactrin. The vomition was once off after both doses and did not persist.

6.6 URINE CORTISOL/CREATININE RATIOS

The cats were followed up for a twelve-month period after the ACTH stimulation tests. Urine was obtained in their home environments, twelve months after the ACTH stimulation tests. Urine cortisol/creatinine ratios had a mean value of 3.3×10^{-6} (range $0.85 - 8.67 \times 10^{-6}$) (Figure 5).

Table 2. Serum cortisol concentrations in 15 cats showing basal cortisol (time 0) and post stimulation cortisol concentrations (at 60, 120 and 180 minutes). Individual peak serum cortisol concentrations are in bold.

Number	Dose of ACTH	0 minutes	60 minutes	120 minutes	180 minutes
Cat 1	125 250	163 193	237 239	**240** **265**	114 168
Cat 2	125 250	187 211	269 263	**274** **294**	161 243
Cat 3	125 250	280 242	**375** **387**	243 335	93 144
Cat 4	125 250	200 133	285 297	**290** **356**	138 218
Cat 5	125 250	178 173	**199** 214	197 **222**	105 194
Cat 6	125 250	214 225	230 237	262 **262**	**263** 259
Cat 7	125 250	147 199	243 282	282 324	**329** **329**
Cat 8	125 250	243 219	**383** 329	197 408	61 **421**
Cat 9	125 250	237 224	**316** **315**	308 311	94 186
Cat 10	125 250	181 106	225 149	**225** 166	161 **173**
Cat 11	125 250	299 122	397 330	**446** **366**	269 263
Cat 12	125 250	354 305	467 487	533 504	**545** **514**
Cat 13	125 250	81 86	239 193	**257** **216**	176 190
Cat 14	125 250	308 167	**351** 258	338 **272**	171 150
Cat 15	125 250	174 236	418 351	**469** 416	313 **421**

Figure 2. Results of serum cortisol concentrations (nmol/l) (y-axis) in 15 cats at time 0 and at 60, 120 and 180 minutes (x-axis) following administration of 125 μg of intravenous synthetic ACTH (tetracosactrin).

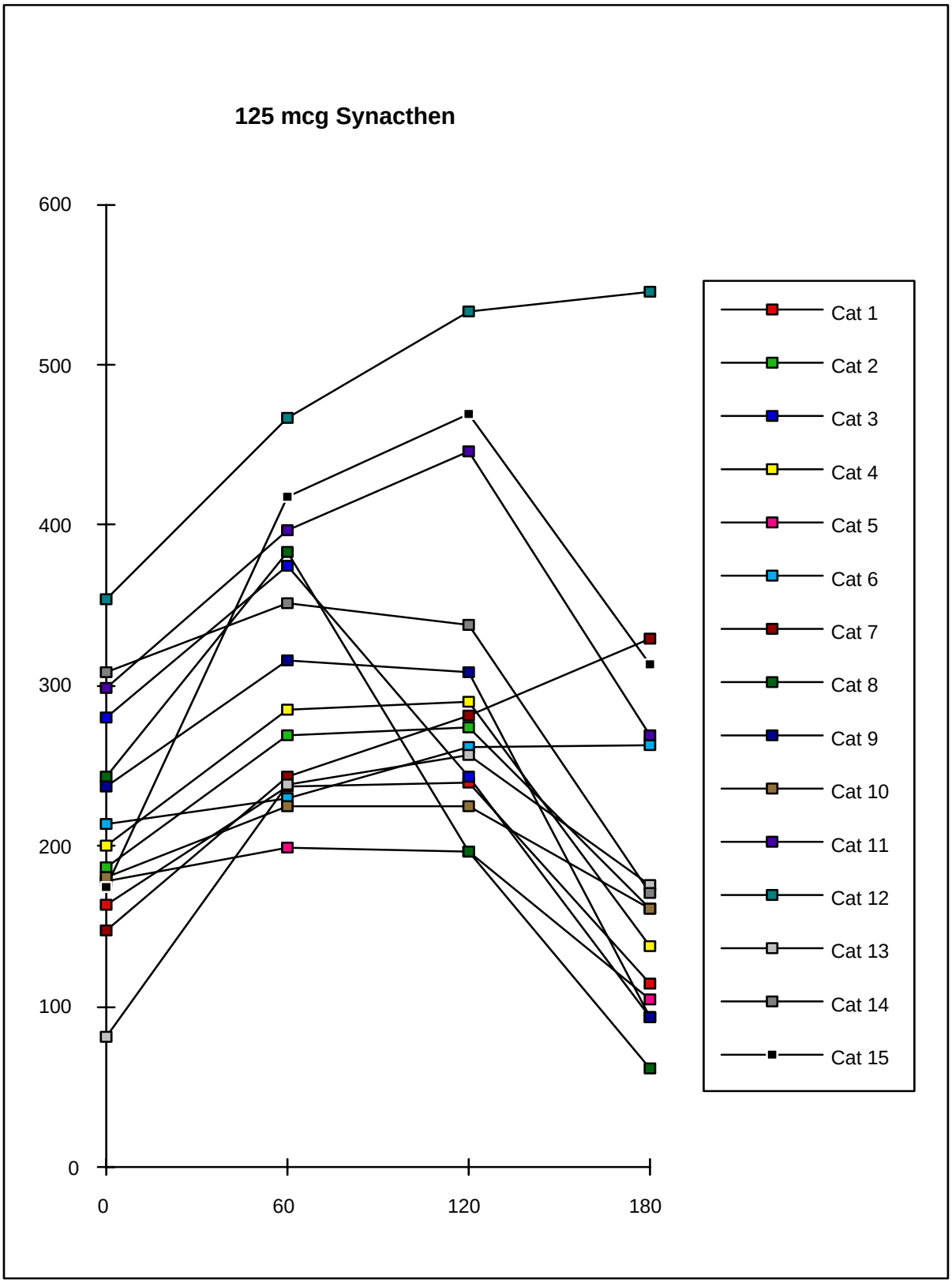


Figure 3. Results of serum cortisol concentrations (nmol/l) (y-axis) in 15 cats at time 0 and at 60, 120 and 180 minutes (x-axis) following administration of 250 µg of intravenous synthetic ACTH (tetracosactrin).

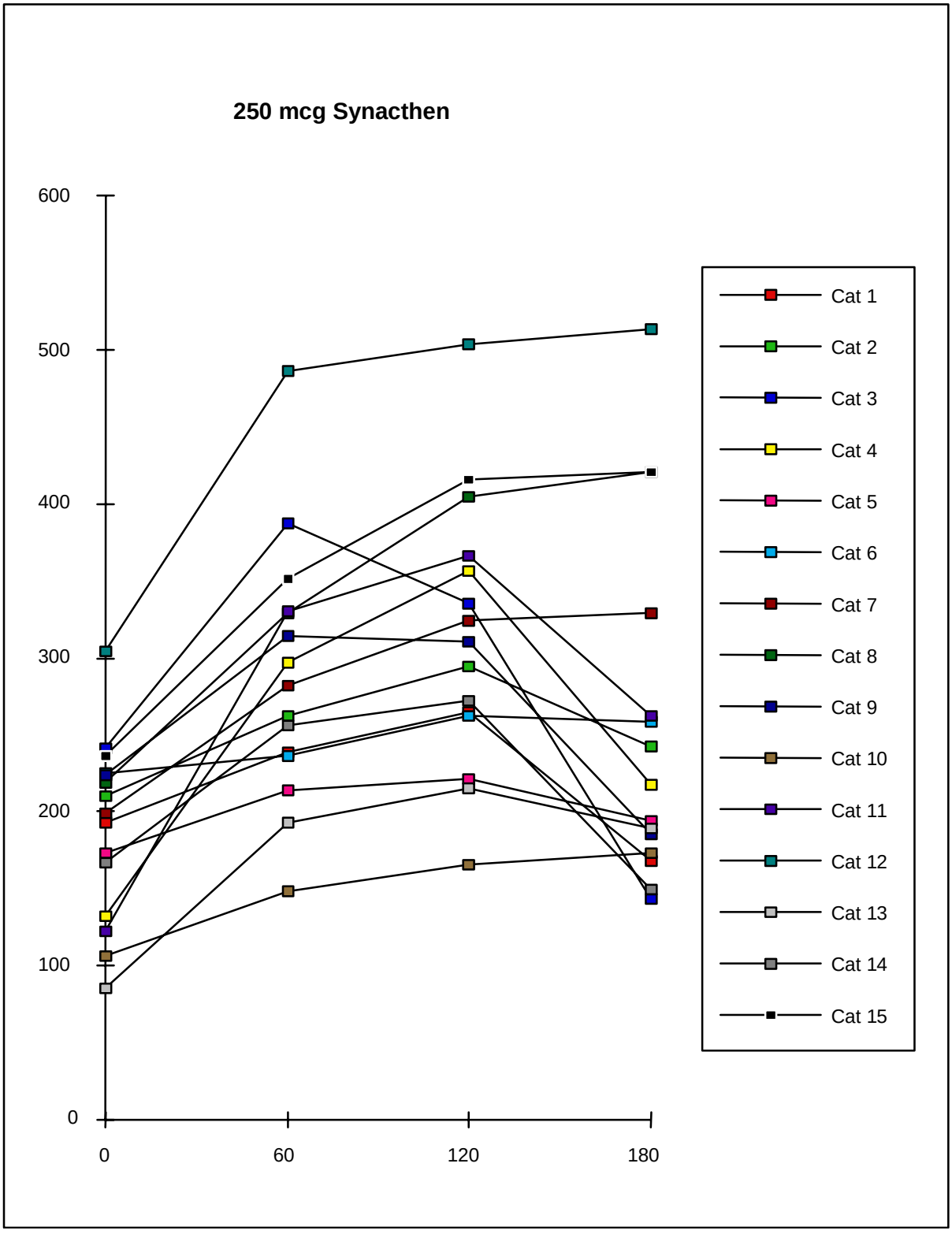
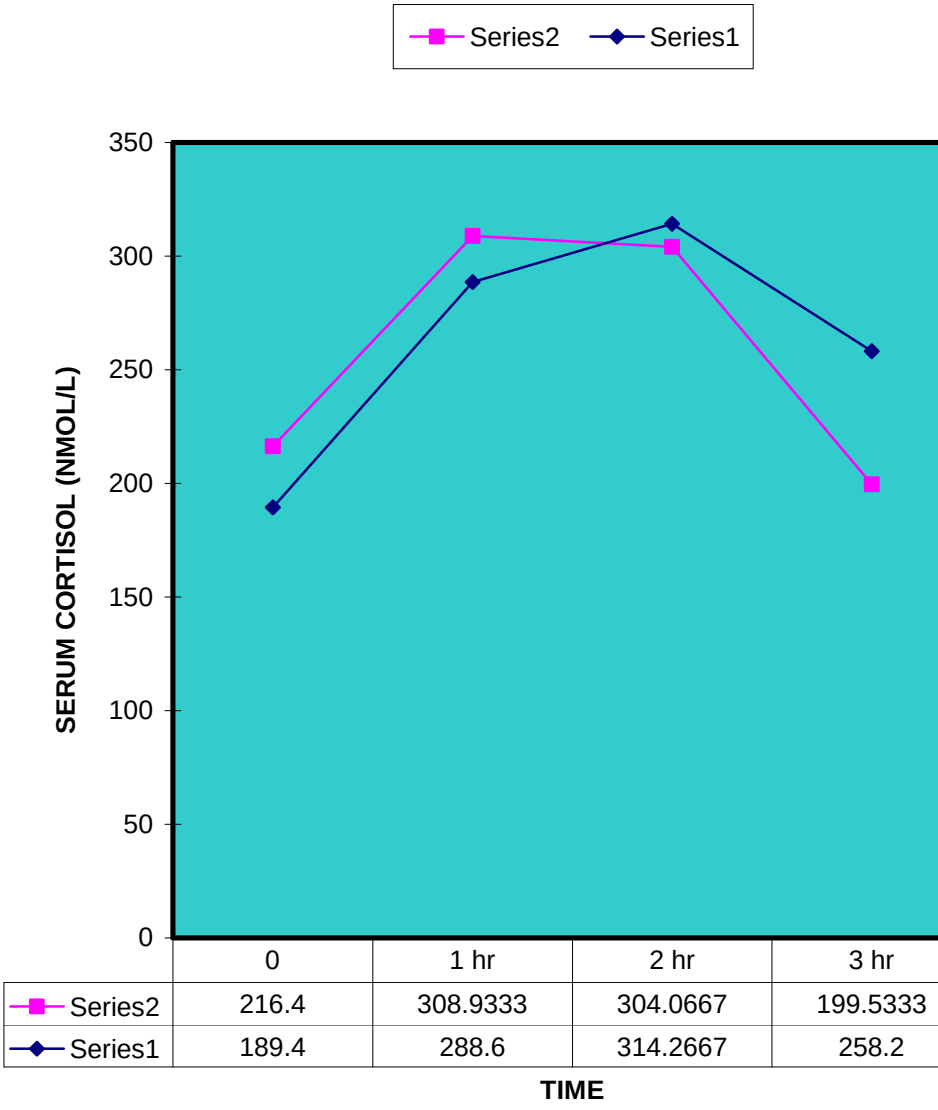
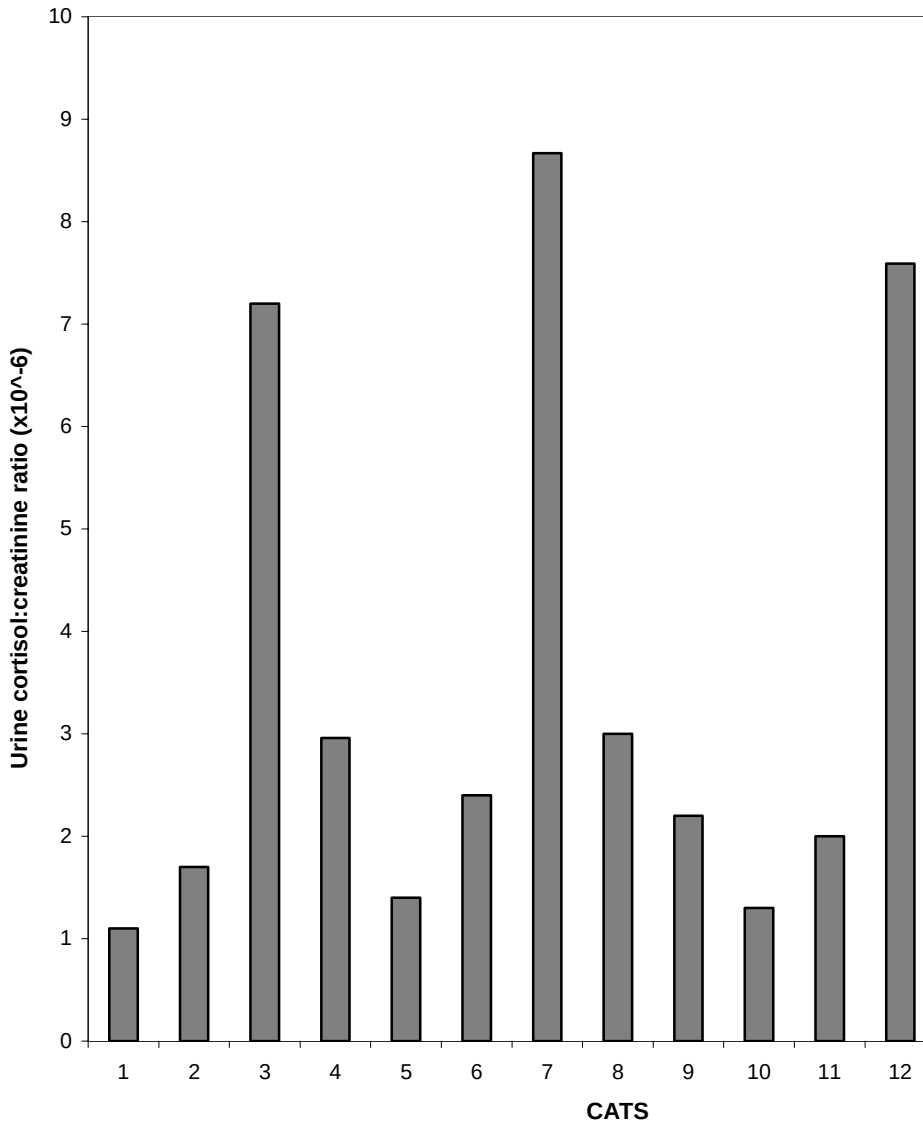


Figure 4: Serum cortisol responses of 15 cats to two doses of tetracosactrin. Mean values of the two different doses at the various time intervals depicted graphically. Means included in the series legends. Series 1 depicts the 125µg dose and series 2 depicts the 250 µg dose.



	0	1 hr	2 hr	3 hr
Series2	216.4	308.9333	304.0667	199.5333
Series1	189.4	288.6	314.2667	258.2

Figure 5. Urinary cortisol/creatinine ratios in 15 cats obtained twelve months after the ACTH stimulation tests.



7. DISCUSSION

The 15 clinic-based, middle-aged to older, normal, but overweight cats used in this study all showed a cortisol response to intravenous synthetic ACTH administration, with similar peak serum cortisol concentrations to those of laboratory conditioned cats previously reported (Peterson and Kemppainen 1992a, Peterson and Kemppainen 1992b, Sparkes and others 1990). The mean serum cortisol concentration of 308 nmol/l at 120 minutes attained by the cats in this study, following the 125 μg dose of tetracosactrin, concurs fairly well and lies mid-way between the values described in the two previous reports using the same preparation and route of administration. Sparkes and others (1990) and Peterson and Kemppainen (1992b) showed a mean serum cortisol concentration of 368 nmol/l and 269 nmol/l respectively at 120 minutes.

The percentage stimulation achieved from basal to peak serum cortisol concentration was between 12 and 217 %. In this respect the results of this study differ markedly from the two previous studies in which intravenous tetracosactrin was used (Sparkes and others 1990, Peterson and Kemppainen 1992b). These studies demonstrated an increase in serum concentrations of between 160 and 1360 % and between 250 and 980 %, respectively. The marked difference in basal serum cortisol concentrations between this study and the previous two studies would help to account for the reduced range in percentage increase. The mean basal serum cortisol concentration was 32 nmol/l in the study by Peterson and Kemppainen (1992b) and 93 nmol/l in the study by Sparkes and others (1990) as opposed to 203 nmol/l in this study. In both these previous studies, laboratory conditioned cats were used. Our study was conducted on client owned cats, brought to the veterinary hospital on the

morning of each test procedure. Thus, this situation more closely resembles normal veterinary practice, with the cats being subjected to a mixture of physical and emotional stress, including a car journey and exposure to the strange smells and noises of other animals in the hospital. The stress was compounded by handling and clipping a jugular vein for blood sampling. It is noteworthy that the baseline cortisol obtained in this study is similar to the serum cortisol concentrations obtained by Willemse and others (1993) in laboratory conditioned cats five minutes after handling for intradermal skin testing. Moon (1997) also demonstrated a baseline serum cortisol concentration of 193 nmol/l in six healthy cats prior to anaesthesia.

The lower resting serum cortisol concentration obtained during the second procedure would suggest that a degree of acclimatisation had taken place. This trend has been noted previously by other authors (Willemse and others 1993, Crager and others 1994). This lower basal cortisol concentration prior to the second procedure could have a bearing on the difference in response and a study with a cross over design would have been more powerful.

The two doses of tetracosactrin in this study produced similar cortisol peaks, but the higher dose produced a more prolonged response. This study thus concurs with the difference in the effect of incremental doses, which had been noted previously by Peterson and Kemppainen (1993). They suggested that 125 µg represents a supraphysiologic dose in cats less than 5 kg. The fact that this study demonstrated statistically similar peaks with both doses, tends to agree with this finding, but illustrates that a more prolonged response can nevertheless be obtained with an even higher dose in cats > 5 kg.

The prolonged serum cortisol response described by Sparkes and others (1990) following a 125 µg dose of tetracosactrin, is more in agreement with the response

observed in our study following the 250 µg dose. The cats used by Sparkes and others (1990) had a mean bodyweight of 3.3 kg, whereas the mean bodyweight of the cats in our study was 6.8 kg. Both sets of cats would therefore have received a similar dose rate on a µg/kg basis and this might explain the similarity. The intravenous administration of ACTH in this study did therefore show an appreciable difference between the two doses, which was not apparent after their intramuscular administration in a previous study (Smith and Feldman 1987).

Although the serum cortisol concentration at the 60 and 120 minute time points were statistically similar using either dose, the individual cortisol responses of the cats showed a marked reduction between the 60 and 120 minute time points in at least two cats after the 125 µg dose. This reduction was much less apparent after the 250 ug dose. These findings would support the argument for using a higher dose of tetracosactrin in heavier cats, should post ACTH sampling be delayed for any reason.

The serum cortisol concentrations obtained post ACTH administration in this study were distinct from those of confirmed cases of feline hyperadrenocorticism using the same route and dose of tetracosactrin. The mean peak serum cortisol concentration in this study was 341 nmol/l compared to 886 nmol/l in nine ACTH stimulation tests performed on five confirmed cases of hyperadrenocorticism (Watson and Herrtage 1998).

The mean UCCR of 3.3×10^{-6} (range 0.85 - 8.67), obtained in these cats was similar to the value of 5×10^{-6} (range 2 - 8.4) obtained by Rochlitz (1997) in 9 healthy cats, using the same methodology. It was also distinctly different from the mean UCCR of 122×10^{-6} (range 51 - 272) obtained by Goossens and others (1995) in 6 cats with hyperadrenocorticism. The UCCR has been proven to be a good screening test for hyperadrenocorticism in dogs, in that a normal result rules out

hyperadrenocorticism (Smiley and Peterson 1993) and the results from normal cats have shown no overlap with cats with hyperadrenocorticism (Henry and others 1996, Goossens and others 1995). The lack of development of clinical signs of hyperadrenocorticism over the follow up period, the modest weight loss achieved and the normal urinary cortisol/creatinine ratios would exclude hyperadrenocorticism as a cause of obesity in the cats in this study.

Transient vomiting was observed in one cat in this study and concurs with side effects noted in previous studies (Smith and Feldman 1987, Sparkes and others 1990). Smith and Feldman (1987) observed this side effect after the intramuscular administration of the 250 µg dose and suggested therefore that this should serve as a deterrent to the use of the higher dose. The same cats were, however, not given the 125 µg dose and this questions the assertion that this side effect is solely attributable to the higher dose. Sparkes and others (1990) noted vomiting in three cats following administration of the lower dose of 125 µg of synthetic ACTH intravenously. The cat in our study experienced transient vomiting episodes immediately after the administration of both doses of tetracosactrin. This finding would tend to support an individual sensitivity to the drug, rather than a dose related phenomenon. It is interesting that this cat was one of only two cats that produced a consistent early serum cortisol peak at 60 minutes following both doses.

8. CONCLUSIONS

It is apparent from this study that similar peak serum cortisol concentrations is achieved in both laboratory and clinic conditions after intravenously administered ACTH and that the peak serum concentration reaches a plateau, regardless of the basal serum cortisol concentration and the dose of ACTH used.

In this study the mean basal serum cortisol concentration in 15 client owned cats is 203 nmol/l (range 72 - 334 nmol/l, mean ± 2 S.D)

This study in a clinic-based population indicates that sampling could be done at 60 minutes following either dose of intravenous ACTH. The 250µg dose did however show a more prolonged response and an alternative protocol might be to sample at 120 minutes after this dose.

Furthermore, the side effect of vomition cannot be attributed to the 250µg dose only.

9. REFERENCES:

BRUYETTE, D.S. (1994) Adrenal function testing. In: Consultations in Feline Internal Medicine, 2nd edition, Ed J.R. August, Chapter 17. W.B. Saunders, Philadelphia, pp 129 - 132

CRAGER, C.S., DILLON, A.R., KEMPPAINEN, R.J., BREWER, W.G. & ANGARONA, D.W. (1994) Adrenocorticotropic hormone and cortisol concentrations after corticotropin-releasing hormone stimulation testing in cats administered methylprednisolone. *American Journal of Veterinary Research* **55**, 704 - 709

DALEY, C.A., ZERBE, C.A., SHICK, R.O. ET AL (1993) Use of metyrapone to treat pituitary-dependent hyperadrenocorticism in a cat with large cutaneous wounds. *Journal of the American Veterinary Medical Association* **202**, 956 – 960

DUESBERG, C.A. & PETERSON, M.E. (1997) Adrenal disorders in cats. *Veterinary Clinics of North America: Small Animal Practice* **27**, 321 - 348

DUESBERG, C.A., NELSON, R.W., FELDMAN, E.C., VADEN, S.C. & SCOTT - MONCRIEFF, C. R. (1995) Adrenalectomy for the treatment of hyperadrenocorticism in cats: 10 cases (1988 - 1992). *Journal of the American Veterinary Medical Association* **207**, 1066 – 1070

FOX, J.G. & BEATTY, J.O. (1975) A case report of complicated diabetes mellitus in a cat. *Journal of the American Animal Hospital Association* **11**, 129-134

FURUZAWA, Y., UNE, Y. & NORURA, Y. (1992) Pituitary dependent hyperadrenocorticism in a cat. *Journal of Veterinary Medical Science* **54**, 1201

GOOSSENS, M.M.C., MEYER, H.P., VOORHOUT, G. & SPRANG, E.M.P. (1995) Urinary excretion of glucocorticoids in the diagnosis of hyperadrenocorticism in cats. *Domestic Animal Endocrinology* **12**, 355 - 362

HENRY, C.J, CLARK, T.P., YOUNG, D.W. & SPANO, J.S. (1996) Urine cortisol:creatinine ratio in healthy and sick cats. *Journal of Veterinary Internal Medicine* **10**, 123 - 126

HERRTAGE, M.E. (1998) Canine hyperadrenocorticism. In: *Manual of Small Animal Endocrinology*, 2nd edn. Eds A.G.Torrance, C.T. Mooney. British Small Animal Veterinary Association, Cheltenham. pp 55 -73

IMMINK, W.F.G.A., VAN TOOR, A.J., VOS, J.H., VAN DER LINDE-SIPMAN, J.S. & LUBBERINK, A.A.M.E. (1992) Hyperadrenocorticism in four cats. *Veterinary Quarterly* **14**, 81 - 85

JOHNSTON, S.D. & MATHER, E.C. (1979) Feline plasma cortisol (hydrocortisone) measured by radioimmunoassay. *American Journal of Veterinary Research* **40**, 190-192

JONES, C.A., REFSAL, K.R., STEVENS, B.J. ET AL (1992) Adrenocortical adenocarcinoma in a cat. *Journal of the American Animal Hospital Association* **28**, 59

KEMPPAINEN, R.J., MANSFIELD, P.D. & SARTIN, J.L. (1984) Endocrine responses of normal cats to TSH and synthetic ACTH administration. *Journal of the American Animal Hospital Association* **20**, 737-740

KIPPERMAN, B.S., NELSON, R.W., GRIFFEY, S.M. ET AL (1992) Diabetes mellitus and exocrine pancreatic neoplasia in two cats with hyperadrenocorticism. *Journal of the American Animal Hospital Association* **28**, 415-418

LAFLAMME, D. (1997) Development and validation of a body condition score system for cats: A clinical tool. *Feline Practice* **25**, 13 - 18

MEDLEAU, L., COWAN, L.A., CORNELIUS, L.M. ET AL (1987) Adrenal function testing in the cat: The effect of low dose intravenous dexamethasone administration. *Research in Veterinary Science* **42**, 260-264

MEIJER, J.J., LUBBERICK, A.A.M.E. & GRUYS, E. (1978) Cushing's syndrome due to adrenocortical adenoma in a cat. *Tijdschrift voor Diergeneeskunde* **103**, 1048 – 1051

MOLLER, D.E. & FLIER, J.S. (1991) Insulin resistance: Mechanisms, syndromes and implications. *New England Journal of Medicine* **325**, 938 - 945

MOON, P. F. (1997) Cortisol suppression in cats after induction of anaesthesia with etomidate, compared with ketamine diazepam combination. *American Journal of Veterinary Research* **58**, 868 – 871

MOONEY, C.T. (1998) Unusual endocrine disorders in the cat. *In Practice* **20**, 345-349

MYERS, N.C. & BRUYETTE, D.S. (1994) Feline adrenocortical disease. Part 1 - Hyperadrenocorticism. *Seminars in Veterinary Medicine and Surgery* **9**, 137 - 143

NELSON, R.W. (1991) Feline Cushing's syndrome. In: Consultations in Feline Internal Medicine Ed J.R. August, WB Saunders, Philadelphia: pp 267-270

NELSON, R.W., FELDMAN, E.C. & SMITH, M.C. (1988) Hyperadrenocorticism in cats: Seven cases (1978 - 1987). *Journal of the American Veterinary Medical Association* **193**, 245 - 250

PETERSON, M.E. (1988) Endocrine disorders in cats: Four emerging diseases. *Compendium on Continuing Education for the Practicing Veterinarian* **10**, 1353 – 1363

PETERSON, M.E. & GRAVES, T.K. (1988) Effects of low dosage of intravenous dexamethasone on serum cortisol concentrations in the normal cat. *Research in Veterinary Science* **44**, 38-40

PETERSON, M.E. & KEMPPAINEN, R.J. (1992a) Comparison of intravenous and intramuscular routes of administering cosyntropin for corticotropin stimulation testing in cats. *American Journal of Veterinary Research* **53**, 1392-1395

PETERSON, M.E. & KEMPPAINEN, R.J. (1992b) Comparison of the immunoreactive plasma corticotropin and cortisol responses to two synthetic corticotropin preparations (tetracosactrin and cosyntropin) in healthy cats. *American Journal of Veterinary Research* **53**, 1752 - 1755

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

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

PETERSON, M.E. & STEELE, P. (1986) Pituitary-dependent hyperadrenocorticism in a cat. *Journal of the American Veterinary Medical Association* **189**, 680 – 683

ROCHLITZ, I. (1997) Welfare of cats in quarantine kennels. Thesis for the Doctorate of Philosophy. Department of Veterinary Clinical Studies, University of Cambridge

ROTHUIZEN, J., REUL, J.M.H.M., VAN SLUIS, F.J., MOL, J.A., RIJNBERK, A. & DE KLOET, E.R. (1993) Increased neuroendocrine reactivity and decreased brain mineralocorticoid receptor binding capacity in aged dogs. *Endocrinology* **132**, 161 – 168

SCHWEDES, C.S. (1997) Mitotane (o,p DDD) treatment in a cat with hyperadrenocorticism. *Journal of Small Animal Practice* **38**, 520-524

SCOTT, D.W., MANNING, T.O. & REIMERS, T.W. (1982) Iatrogenic Cushing's syndrome in the cat. *Feline Practice* **12**, 30

SMILEY, L.E. & PETERSON, M.E. (1993) Evaluation of a urine cortisol:creatinine ratio as a screening test for hyperadrenocorticism in dogs. *Journal of Veterinary Internal Medicine* **7**, 163 - 168

SMITH, M.C. & FELDMAN, E.C. (1987) Plasma endogenous ACTH concentrations and plasma cortisol responses to synthetic ACTH and dexamethasone sodium phosphate in healthy cats. *American Journal of Veterinary Research* **48**, 1719 -1724

SPARKES, 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**, 1 – 4

STOLP, R., RIJNBERK, A. & MEIJER, J.C. (1983) Urinary corticoids in the diagnosis of canine hyperadrenocorticism. *Research in Veterinary Science* **34**, 141-144

SWIFT, G.A. & BROWN, R.H. (1976) Surgical treatment of Cushing's syndrome in the cat. *The Veterinary Record* **99**, 374-375

USHER, D.G. (1991) Hyperadrenocorticism in a cat. *Canadian Veterinary Journal* **32**, 326

VALENTINE, R.W. & SILBER, A. (1996) Feline hyperadrenocorticism: a rare case. *Feline Practice* **24**, 6

VAN SLUIJS, F.J. & SJOLLEMA, B.E. (1992) Adrenalectomy in 36 dogs and 2 cats with hyperadrenocorticism. *Tijdschrift voor Diergeneeskunde* **117**, 29

WATSON, A.D.J., CHURCH, D.B., EMSLIE, D.R. & MIDDLETON, D.J. (1989) Comparative effects of proligestone and megesterol acetate on basal plasma glucose concentrations and cortisol responses to exogenous adrenocorticotrophic hormone in cats. *Research in Veterinary Science* **46,** 374-376

WATSON, P.J. & HERRTAGE, M.E. (1998) Hyperadrenocorticism in six cats. *Journal of Small Animal Practice* **39**, 175 -179

WILLEMSE, T., VROOM, M.W., MOL, J.A. & RIJNBERK, A. (1993) Changes in plasma cortisol, corticotropin and alpha-melanocyte-stimulating hormone concentrations in cats before and after physical restraint and intradermal skin testing. *American Journal of Veterinary Research* **54**, 69 - 72

ZERBE, C.A. (1989) Feline Hyperadrenocorticism. In: Kirk's Current Veterinary Therapy X, Ed R.W. Kirk, W.B. Saunders, Philadelphia, pp 1038 - 1045

ZERBE, C.A., NACHREINER, R.F., DUNSTAN, R.W. ET AL (1987) Hyperadrenocorticism in a cat. *Journal of the American Veterinary Medical Association* **190**, 559-562

10. APPENDIX - JOURNAL ARTICLE

Cortisol response to two different doses of intravenous synthetic ACTH (tetracosactrin) in overweight cats

Fifteen middle-aged to older, overweight cats attending a first-opinion clinic were investigated to rule out hyperadrenocorticism as a cause of their weight problem, using two different protocols for the adrenocorticotropic hormone (ACTH) stimulation test. The cats received intravenous synthetic ACTH (tetracosactrin) at an initial dose of 125 µg; a second test was performed between two and three weeks later, using a dose of 250 µg intravenously. The mean basal serum cortisol concentration was 203 nmol / litre (range 81 to 354 nmol / litre). The highest mean serum cortisol concentration occurred at 60 minutes following the 125 µg dose and at 120 minutes following the 250 µg dose. There was, however, no statistically significant difference between these peak cortisol concentrations attained using either dose of tetracosactrin. A significantly higher mean serum cortisol concentration was attained after the higher dose at the 180 minutes time point, indicating a more prolonged response when compared with the lower dose. The cats were followed up for one year after the initial investigations and none were found to develop hyperadrenocorticism during this time.

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INTRODUCTION

The adrenocorticotropic hormone (ACTH) stimulation test is a widely accepted diagnostic screening test for adrenocortical abnormalities in both humans and animals. Hyperadrenocorticism (HAC) is a rare endocrine disorder in cats and there are only about 70 cases described in the veterinary literature. Only four small case series of feline HAC have been published (Nelson and others 1988, Immink and others 1992, Duesberg and others 1995, Watson and Herrtage 1998). The clinical signs, clinicopathological and radiological findings in feline HAC are generally less dramatic than in dogs, which might explain why feline cases are diagnosed at a relatively later stage of the disease.

More than 80 per cent of reported cases of feline HAC presented with concurrent diabetes mellitus, which is considered to be a late complication of the disease in dogs (Duesberg and Peterson 1997). In a recent retrospective study of six cases of feline HAC, all cases were described as overweight but, more importantly, three of these cases presented without diabetes mellitus (Watson and Herrtage 1998). The mechanism of diabetes mellitus occurring secondarily to HAC is believed to be due to insulin resistance and the down-regulation of insulin receptors, and HAC cases without diabetes mellitus can reasonably be assumed to be in an earlier stage of the disease. Weight gain and a potbellied appearance are also reported to be among the most frequently recognised signs of HAC in cats (Myers and Bruyette 1994, Duesberg and Peterson 1997).

Various authors have evaluated the ACTH stimulation test in laboratory cats. These studies used a variety of ACTH preparations at different doses, and with different routes of administration and sampling times.

Smith and Feldman (1987) compared the results of the intramuscular ACTH stimulation test using synthetic ACTH (cosyntropin) at a dose of 125 µg in five cats with a dose of 250 µg in six different cats and found no significant difference between responses resulting from the two dosages. All the cats weighed between 3·2 and 5·5 kg. Two of the cats receiving the 250 µg dose vomited and remained depressed for two hours; the authors concluded that the adverse reaction observed should serve as a deterrent to the use of the 250 µg dose in cats.

Sparkes and others (1990) were the first to report on the response of cats to intravenous synthetic ACTH (tetracosactrin) – the preparation used in Europe. They used a dose of 125 µg in 15 barrier-maintained colony cats weighing between 2·6 and 4·7 kg. It was concluded that most of the cats produced peak serum cortisol concentrations at 180 minutes after tetracosactrin.

Peterson and Kemppainen (1992a) compared the intramuscular and intravenous routes of administration, using a

dose of 125 µg of cosyntropin in 10 colony cats of between 2·7 and 5 kg bodyweight. They found that the intravenous route induced significantly greater and more prolonged adrenocortical stimulation than that induced intramuscularly. They also demonstrated a peak serum cortisol response between 60 and 90 minutes after intravenously administered cosyntropin, which was earlier than the peak reported by Sparkes and others (1990).

Peterson and Kemppainen (1992b) then evaluated the cortisol responses to two available synthetic ACTH preparations (tetracosactrin and cosyntropin), administered intravenously in 10 cats, and found that these preparations produced a comparable, if not identical, pattern of response. In that study they noted that four cats which responded late to one preparation also responded late to the other. This led to a change in their recommendation of sampling times after intravenous administration of ACTH to include not only a 60 to 90 minute sample, but also a sample at 120 to 180 minutes to ensure that the peak response was not missed.

Peterson and Kemppainen (1993) evaluated the serum cortisol responses after the intravenous administration of incremental doses of cosyntropin (1·25, 12·5 and 125 µg) to six normal cats weighing between 3 and 5 kg. They demonstrated comparable peak concentrations of serum cortisol at all three doses, but a more prolonged stimulation using the highest dose.

In six studies in healthy cats in which synthetic ACTH (cosyntropin or tetracosactrin) was administered intravenously, 125 µg was the highest dose used. All the cats weighed less than 5·3 kg, but no mention was made of their respective body condition scores (Peterson and others 1984, Sparkes and others 1990, Peterson and Kemppainen 1992a,b, 1993, Crager and others 1994). The protocol for ACTH stimulation testing in dogs recommends a dose of 125 µg of tetracosactrin intravenously for dogs of less than 5 kg and 250 µg for dogs over 5 kg bodyweight (Herrtage 1998).

All previous studies of intravenous ACTH stimulation testing in cats have been carried out in laboratory conditioned animals and this may not be truly representative of the responses seen in a clinic-based population. In addition, some authors have demonstrated increased hypothalamic-pituitary-adrenal activity in older animals, casting doubt on the validity of data obtained using young cats. Goossens and others (1995) demonstrated increased urinary excretion of glucocorticoids with increasing age in cats. This is also true for dogs, increased activity of the hypothalamic-pituitary-adrenal axis being demonstrated in older animals, resulting in higher plasma concentrations of ACTH and cortisol, and higher urine cortisol/creatinine ratios (UCCRs) (Rothuizen and others 1993).

The aim of the present study was therefore to assess the cortisol responses to intravenously administered ACTH (tetracosactrin) in a middle-aged to older, clinic-based population of cats, and to determine whether any benefit could be derived from using the 250 µg dose in cats over 5 kg bodyweight.

MATERIALS AND METHODS

Fifteen healthy cats (six neutered females and nine neutered males) of more than 5 kg bodyweight were recruited. The animals were identified from the records of the obesity clinic run at a first opinion veterinary hospital in Hertfordshire. The cats had failed to lose weight despite appropriate dietary advice given to their owners by the obesity clinic staff. They were therefore investigated to exclude early HAC as a cause of their lack of weight loss. Other abnormalities were excluded based on the results of a full clinical examination, including a haematological and biochemical analysis. The clinical case histories were carefully scrutinised to ensure that none of the cats had received any medication in the six months preceding the study.

Each of these cats was awarded a body condition score based on a nine-point score system described by Laflamme (1997) and were only included in the study if they were considered overweight (body condition score of 6 or greater).

The cats were examined regularly at the obesity clinic for a 12-month period following the ACTH stimulation tests. In order to exclude HAC further, urine samples were obtained for UCCRs 12 months after the ACTH stimulation tests.

Protocol

ACTH stimulation test procedures were started between 9 and 10 am. Blood samples were obtained by repeated jugular venepuncture. On the morning of the first test, each cat was given 125 µg of synthetic ACTH (tetracosactrin) intravenously. Blood samples were obtained immediately before and at 60, 120 and 180 minutes after injection of tetracosactrin. The results of the first test were analysed and a second ACTH stimulation test was performed only if the results of the first test were within currently accepted normal limits (Sparkes and others 1990). After an interval of at least two weeks (range two to three weeks) a second ACTH stimulation test was performed using a dose of 250 µg tetracosactrin intravenously. Blood samples were collected by repeated jugular venepuncture at the same time intervals.

The samples were centrifuged within two hours of collection. The serum was harvested and stored at –18°C until assayed. Serum cortisol concentrations were measured by radioimmunoassay. The inter-assay variation across the range 54 to 927 nmol/litre was calculated to be 5·8 to 7·8 per cent. The intra-assay variation across the range 97 to 1040 nmol/litre was calculated to be 4·7 to 7·9 per cent. The lower limit of quantification was calculated to be 20 nmol/litre.

Urine was obtained from 10 of the cats by cystocentesis in their home environment and from the remaining five cats by free

flow samples obtained from the litter tray in the home, using non-absorbent aquarium gravel (Aquagravel; Madingley Mulch, Cambridge) as litter. Urine was collected in plastic syringes or into plastic serum blood tubes and frozen within 24 hours at –18°C until assayed. Urinary cortisol was measured using a radioimmunoassay test kit (Corti-Cote; Cortisol Solid Phase Component System, Becton Dickinson, Oxford). Urinary creatinine concentrations were determined by spectrophotometry (Jaffe reaction) on an autoanalyser (Beckman CX5CE; Beckman Coulter, High Wycombe). The urinary cortisol intra-assay variation across the range of 97 to 1040 nmol/litre was 4·7 to 7·9 per cent and the inter-assay variation across the range of 91 to 1090 nmol/litre was 6·6 to 7·4 per cent. The urinary creatinine had a intra- and inter-assay coefficient of variation of less than 0·1 per cent across the range 8206 to 33,951 µmol/litre.

Statistical analysis of the results was performed as follows: normally distributed data was tested using the paired Student's *t*-test; otherwise the Wilcoxon signed rank test was used. A P value of 0·05 or less was considered significant.

RESULTS

The mean weight of the 15 cats was 6·8 kg (range 5·1 to 9 kg). The mean age of the cats was eight years (range five to 11 years). The mean body condition score was 7·7 (range 6 to 9).

The results of the serum cortisol concentrations of individual cats are shown in Table 1 and in Figs 1 and 2. The basal serum cortisol concentrations for both ACTH stimulation test procedures (n=30) ranged from 81 to 354 nmol/litre (mean 203 nmol/litre). The mean basal serum cortisol concentration was 216 nmol/litre at the start of the first procedure and 189 nmol/litre before the second procedure. This difference was not statistically significant (Student's *t*-test, P = 0·14).

Serum cortisol concentrations increased above the basal concentration following the administration of either dose of tetracosactrin. The time to reach peak serum cortisol concentration was variable and tended to occur earlier following the lower dose. Following the 125 µg dose, six out of 15 cats (40 per cent) peaked at 60 minutes, six cats (40 per cent) peaked at 120 minutes and three (20 per cent) peaked at 180 minutes. Following the 250 µg dose, two out of 15 cats (13 per cent) peaked at 60 minutes, eight (53 per cent) peaked at 120 minutes and five cats (33 per cent) peaked at 180 minutes.

All cats had a marked cortisol response to tetracosactrin at 60 minutes. The two doses of tetracosactrin produced statistically similar cortisol results at both 60 and 120 minutes. The mean serum cortisol concentrations at 60 minutes for the 125 and 250 µg doses were 308 and 288 nmol/litre, respectively, and at 120 minutes the concentrations were 304 nmol/litre for the 125 µg dose and 314 nmol/litre for the 250 µg dose. The mean of the peak serum cortisol concentrations in the 15 cats was 331 nmol/litre (range 199 to 545 nmol/litre) following the 125 µg dose and 321 nmol/litre (range 173 to 514 nmol/litre) following the 250 µg dose.

However, analysis of the individual cortisol responses showed that two cats had a marked reduction in their serum cortisol concentrations at between 60 and 120

Table 1. Serum cortisol concentrations in 15 cats showing basal cortisol (time 0) and poststimulation cortisol concentrations (at 60, 120 and 180 minutes) following intravenous administration of two different tetracosactrin (synthetic ACTH) doses

Cat	Dose of ACTH	0	60	120	180
1	125	163	237	**240**	114
	250	193	239	**265**	168
2	125	187	269	**274**	161
	250	211	263	**294**	243
3	125	280	**375**	243	93
	250	242	**387**	335	144
4	125	200	285	**290**	138
	250	133	297	**356**	218
5	125	178	**199**	197	105
	250	173	214	**222**	194
6	125	214	230	262	**263**
	250	225	237	**262**	259
7	125	147	243	282	**329**
	250	199	282	324	**329**
8	125	243	**383**	197	61
	250	219	329	405	**421**
9	125	237	**316**	308	94
	250	224	**315**	311	186
10	125	181	**225**	**225**	161
	250	106	149	166	**173**
11	125	299	397	**446**	269
	250	122	330	**366**	263
12	125	354	467	533	**545**
	250	305	487	504	**514**
13	125	81	239	**257**	176
	250	86	193	**216**	190
14	125	308	**351**	338	171
	250	167	256	**272**	150
15	125	174	418	**469**	313
	250	236	351	416	**421**

Figures in bold represent the peak serum cortisol concentration achieved with each dose

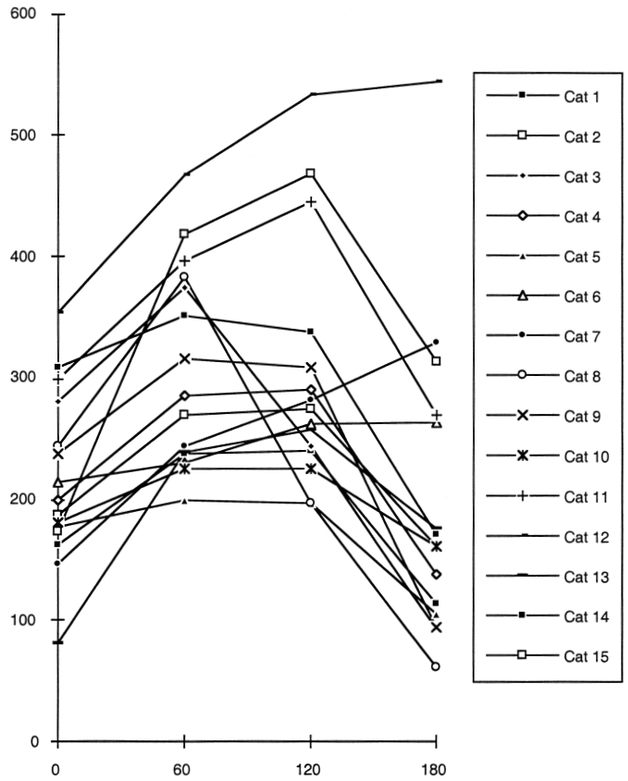

FIG 1. Results of serum cortisol concentrations in 15 cats at time 0 and at 60, 120 and 180 minutes following administration of 125 µg of intravenous synthetic ACTH (tetracosactrin)

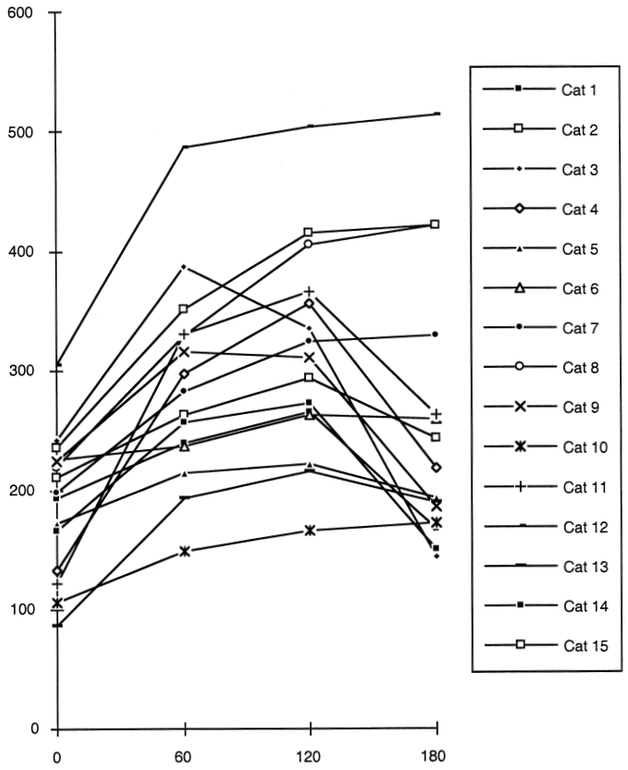

FIG 2. Results of serum cortisol concentrations in 15 cats at time 0 and at 60, 120 and 180 minutes following administration of 250 µg of intravenous synthetic ACTH (tetracosactrin)

minutes following the 125 µg dose, which was not as apparent following the 250 µg dose (Figs 1 and 2). In addition, the median serum cortisol concentration at 180 minutes was significantly higher following the 250 µg dose (218 nmol/litre) than it was following the 125 µg dose (161 nmol/litre) (Wilcoxon signed rank test, P = 0·013), indicating a more prolonged response following stimulation with the 250 µg dose of tetracosactrin (Fig 3).

Nine out of 15 cats (60 per cent) peaked at similar times during both procedures. The three cats that peaked at 180 minutes following the 125 µg dose also peaked at 180 minutes following the 250 µg dose. This would indicate that they were consistent late responders. The five cats that peaked at 180 minutes following the 250 µg dose had peak values that did not differ significantly from their values at 120 minutes, indicating that stimulation had probably reached a plateau.

The percentage increase in serum cortisol over baseline values ranged from 12 to 217 per cent (mean 63 per cent) following the 125 µg dose and from 16 to 200 per cent (mean 78 per cent) following the 250 µg dose.

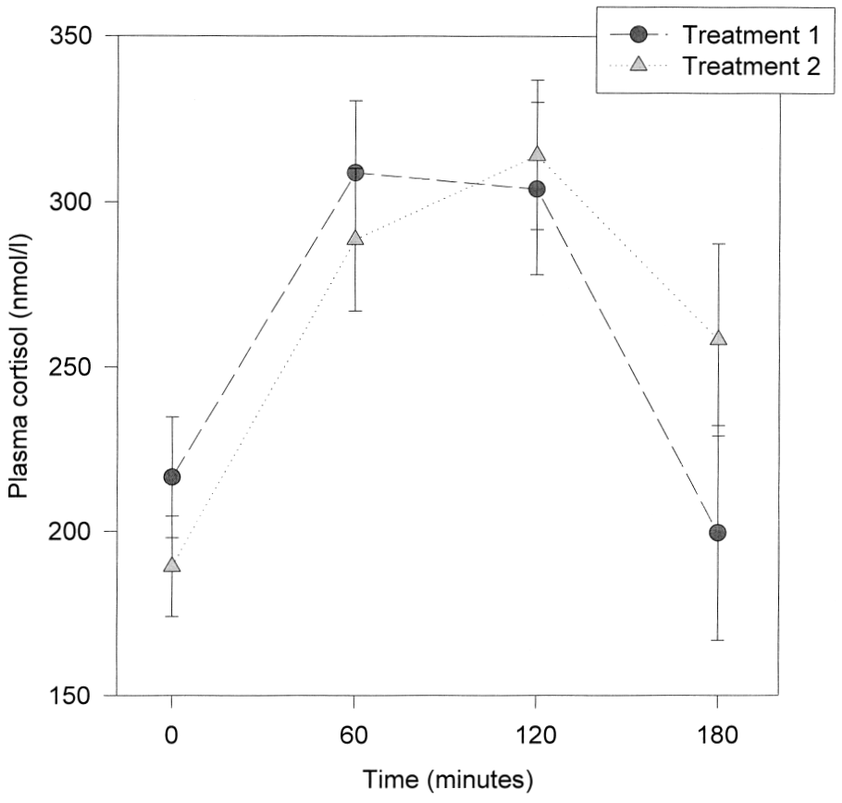

FIG 3. Mean serum cortisol responses (± SD) to 125 µg (treatment 1) and 250 µg (treatment 2) of intravenous synthetic ACTH

One cat showed transient vomiting. This occurred shortly after the intravenous administration of both the 125 and 250 µg doses of tetracosactrin.

All cats were followed up with regular weight checks and re-enforcement of the dietary programme over the following year. A mean reduction in bodyweight of 6 per cent (range 0 to 14 per cent) was achieved during the study. UCCRs obtained 12 months after the ACTH stimulation tests had a mean value of $3{\cdot}3 \times 10^{-6}$ (range $0{\cdot}85$ to $8{\cdot}67 \times 10^{-6}$).

DISCUSSION

The 15 clinic-based, middle-aged to older, normal, but overweight, cats all showed a cortisol response to ACTH administration, with similar peak serum cortisol concentrations to those of laboratory conditioned cats previously reported. The mean serum cortisol concentration of 308 nmol/litre at 120 minutes attained by the cats in this study, following the 125 µg dose of tetracosactrin, concurs fairly well and lies midway between the values described in the two previous reports using the same preparation and route of administration. Sparkes and others (1990) and Peterson and Kemppainen (1992b) reported a mean serum cortisol concentration of 368 nmol/litre and 269 nmol/litre, respectively, at 120 minutes.

The percentage increase in serum cortisol concentration over baseline was between 12 and 217 per cent in the present study. In this respect, the results of this study differ markedly from the two previous studies in which intravenous tetracosactrin was used and in which percentage increases over baseline of as high as 1360 per cent were achieved (Sparkes and others 1990, Peterson and Kemppainen 1992b).

The marked difference in basal serum cortisol concentrations between this study and the previous two studies would help to account for the reduced range in percentage increase. The mean basal serum cortisol concentration was 32 nmol/litre in the study by Peterson and Kemppainen (1992b) and 79·8 nmol/litre in the study by Sparkes and others (1990), as opposed to 203 nmol/litre in the present study. In both these previous studies, laboratory conditioned cats were used. The present study was conducted on client-owned cats, brought to the veterinary hospital on the morning of each test procedure. This situation more closely resembles normal veterinary practice, with the cats being subjected to a mixture of physical and emotional stress, including a car journey and exposure to the strange smells and noises of other animals in the hospital. The stress was then compounded by handling and clipping of a jugular vein to facilitate blood sampling. It is noteworthy that the baseline cortisol obtained in this study is similar to the serum cortisol concentrations obtained by Willemse and others (1993) in laboratory conditioned cats five minutes after handling for intradermal skin testing. Moon (1997) also demonstrated a similar baseline serum cortisol concentration of 193 nmol/litre in six healthy cats before anaesthesia.

The two doses of tetracosactrin in this study produced similar cortisol peaks, but the higher dose tended to produce a more prolonged response. This finding concurs with the cortisol responses to incremental doses, which had been noted previously by Peterson and Kemppainen (1993). They suggested that 125 µg represents a supraphysiological dose in cats of less than 5 kg bodyweight. The fact that the present authors have demonstrated statistically similar peaks with both doses tends to agree with this finding, but illustrates that a more prolonged response can nevertheless be obtained with an even higher dose in cats of more than 5 kg bodyweight. The prolonged serum cortisol response described by Sparkes and others (1990) following a 125 µg dose of tetracosactrin is more in agreement with the response observed in the present study following the 250 µg dose. The cats used by Sparkes and others (1990) had a mean bodyweight of 3·3 kg, whereas the mean bodyweight of the cats in the present study was 6·8 kg. Both sets of cats would therefore have received a similar dose rate on a µg/kg basis and this might explain the similarity. The intravenous administration of ACTH in this study did therefore show an appreciable difference between the two doses, which was not apparent after their intramuscular administration in a previous study (Smith and Feldman 1987).

Although the serum cortisol concentrations at the 60 and 120 minute time points were statistically similar using either dose in the present study, the individual cortisol responses of the cats showed a marked reduction between the 60 and 120 minute time points in two cats after the 125 µg dose. This reduction was less marked after the 250 µg dose. These findings would support the argument for using a higher dose of tetracosactrin in heavier cats, especially if sampling is likely to be delayed for any reason.

Transient vomiting was observed in one cat in this study and concurs with side effects noted in previous studies (Smith and Feldman 1987, Sparkes and others 1990). Smith and Feldman (1987) observed this side effect after the intramuscular administration of the 250 µg dose and suggested therefore that this should serve as a deterrent for the use of the higher dose. The same cats were, however, not given the 125 µg dose and this questions the assertion that this side effect is solely attributable to the higher dose. Sparkes and others (1990) noted vomiting in three cats following administration of the lower dose of 125 µg of synthetic ACTH intravenously. The cat in the present study experienced transient vomiting episodes immediately after the administration of both doses of tetracosactrin. This finding would tend to support an individual sensitivity to the drug, rather than a dose-related phenomenon. It is interesting that this cat was one of only two cats that produced a consistent early serum cortisol peak at 60 minutes following both of the doses.

The serum cortisol concentrations

obtained post-ACTH administration in this study were distinct from those of confirmed cases of feline HAC using the same dose and route of administration of tetracosactrin. The mean peak serum cortisol concentration in this study was 341 nmol/litre compared with 886 nmol/litre in nine ACTH stimulation tests performed on five confirmed cases of HAC (Watson and Herrtage 1998).

The mean UCCR of $3{\cdot}3 \times 10^{-6}$ (range 0·85 to 8·67) obtained in these cats was similar to the value of 5×10^{-6} (range 2 to $8{\cdot}4 \times 10^{-6}$) obtained by Rochlitz (1997) for nine healthy cats, using the same methodology, and is distinct from the mean UCCR of 122×10^{-6} (range 51 to 272×10^{-6}) obtained by Goossens and others (1995) for six cats with HAC. The UCCR has been proven to be a good screening test for HAC in dogs in that a normal result rules out the condition (Smiley and Peterson 1993), and the fact that the results from normal cats have shown no overlap with cats with HAC (Goossens and others 1995, Henry and others 1996). The lack of development of clinical signs of HAC over the follow-up period, the modest weight loss achieved and the normal UCCRs would exclude HAC as a cause of obesity in the cats in the present study.

Conclusions

In this study, which used middle-aged to older, overweight, clinic-based cats, the mean basal serum cortisol concentration was 203 nmol/litre (range 81 to 354 nmol/litre). This concentration was considerably higher than basal serum cortisol concentrations reported in studies using laboratory cats. Following intravenous administration of synthetic ACTH, the cats in this study produced similar peak serum cortisol concentrations to those reported in previous studies.

This study would indicate that a single serum sample collected at 60 minutes following intravenous tetracosactrin would be appropriate and that no significant difference between the doses of ACTH could be determined at this time point. It also supports previous reports which suggested that higher doses of ACTH did not increase the absolute cortisol concentrations, but did tend to produce a more prolonged response. A potential benefit of using 250 µg of tetracosactrin intravenously in cats over 5 kg would be to take advantage of this more prolonged response, ensuring that adequate cortisol concentrations are achieved even if sampling is delayed for any reason.

Vomiting would appear to be a rare side effect following intravenous administration of tetracosactrin and would not appear to be dose related at the dose rates used in this study.

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References

CRAGER, C. S., DILLON, A. R., KEMPPAINEN, R. J., BREWER, W. G. & ANGARONA, D. W. (1994) Adrenocorticotropic hormone and cortisol concentrations after corticotropin-releasing hormone stimulation testing in cats administered methylprednisolone. *American Journal of Veterinary Research* **55**, 704-709

DUESBERG, C. A., NELSON, R. W., FELDMAN, E. C., VADEN, S. C. & SCOTT-MONCRIEFF, C. R. (1995) Adrenalectomy for the treatment of hyperadrenocorticism in cats: 10 cases (1988-1992). *Journal of the American Veterinary Medical Association* **207**, 1066-1070

DUESBERG, C. A. & PETERSON, M. E. (1997) Adrenal disorders in cats. *Veterinary Clinics of North America: Small Animal Practice* **27**, 321-348

GOOSSENS, M. M. C., MEYER, H. P., VOORHOUT, G. & SPRANG, E. M. P. (1995) Urinary excretion of glucocorticoids in the diagnosis of hyperadrenocorticism in cats. *Domestic Animal Endocrinology* **12**, 355-362

HENRY, C. J., CLARK, T. P., YOUNG, D. W. & SPANO, J. S. (1996) Urine cortisol:creatinine ratio in healthy and sick cats. *Journal of Veterinary Internal Medicine* **10**, 123-126

HERRTAGE, M. E. (1998) Canine hyperadrenocorticism. In: Manual of Small Animal Endocrinology, 2nd edn. Eds A. G. Torrance and C. T. Mooney. British Small Animal Veterinary Association, Cheltenham. pp 55-73

IMMINK, W. F. G. A., VAN TOOR, A. J., VOS, J. H., VAN DER LINDE-SIPMAN, J. S. & LUBBERINK, A. A. M. E. (1992) Hyperadrenocorticism in four cats. *Veterinary Quarterly* **14**, 81-85

LAFLAMME, D. (1997) Development and validation of a body condition score system for cats: a clinical tool. *Feline Practice* **25**, 13-18

MOON, P. F. (1997) Cortisol suppression in cats after induction of anaesthesia with etomidate, compared with ketamine diazepam combination. *American Journal of Veterinary Research* **58**, 868-871

MYERS, N. C. & BRUYETTE, D. S. (1994) Feline adrenocortical disease. Part 1 – hyperadrenocorticism. *Seminars in Veterinary Medicine and Surgery* **9**, 137-143

NELSON, R. W., FELDMAN, E. C. & SMITH, M. C. (1988) hyperadrenocorticism in cats: seven cases (1978-1987). *Journal of the American Veterinary Medical Association* **193**, 245-250

PETERSON, M. E. & KEMPPAINEN, R. J. (1992a) Comparison of intravenous and intramuscular routes of administering cosyntropin for corticotropin stimulation testing in cats. *American Journal of Veterinary Research* **53**, 1392-1395

PETERSON, M. E. & KEMPPAINEN, R. J. (1992b) Comparison of the immunoreactive plasma corticotropin and cortisol responses to two synthetic corticotropin preparations (tetracosactrin and cosyntropin) in healthy cats. *American Journal of Veterinary Research* **53**, 1752-1755

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

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

ROCHLITZ, I. (1997) Welfare of cats in quarantine kennels. PhD Thesis. University of Cambridge

ROTHUIZEN, J., REUL, J. M. H. M., VAN SLUIS, F. J., MOL, J. A., RIJNBERK, A. & DE KLOET, E. R. (1993) Increased neuroendocrine reactivity and decreased brain mineralocorticoid receptor binding capacity in aged dogs. *Endocrinology* **132**, 161-168

SMILEY, L. E. & PETERSON, M. E. (1993) Evaluation of a urine cortisol:creatinine ratio as a screening test for hyperadrenocorticism in dogs. *Journal of Veterinary Internal Medicine* **7**, 163-168

SMITH, M. C. & FELDMAN, E. C. (1987) Plasma endogenous ACTH concentrations and plasma cortisol responses to synthetic ACTH and dexamethasone sodium phosphate in healthy cats. *American Journal of Veterinary Research* **48**, 1719-1724

SPARKES, 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**, 1-4

WATSON, P. J. & HERRTAGE, M. E. (1998) Hyperadrenocorticism in six cats. *Journal of Small Animal Practice* **39**, 175-184

WILLEMSE, T., VROOM, M. W., MOL, J. A. & RIJNBERK, A. (1993) Changes in plasma cortisol, corticotropin and alpha melanocyte-stimulating hormone concentrations in cats before and after physical restraint and intradermal testing. *American Journal of Veterinary Research* **54**, 69-72