24 HOUR HORMONE AND SERUM ELECTROLYTE LEVELS OF DOGS WITH PITUITARY-DEPENDENT HYPERADRENO-CORTICISM TREATED WITH TRILOSTANE

CLAUDIA LEHNERT

INAUGURAL-DISSERTATION
zur Erlangung des Grades eines
Dr. med. vet.
beim Fachbereich Veterinärmedizin
der Justus-Liebig-Universität Gießen

édition scientifique
VVB LAUFERSWEILER VERLAG

1. Auflage 2007

1st Edition 2007

Printed in Germany

VVB LAUFERSWEILER VERLAG
édition scientifique

STAUFENBERGRING 15, D-35396 GIESSEN
Tel: 0641-5599888 Fax: 0641-5599890
email: redaktion@doktorverlag.de

www.doktorverlag.de

Aus der Klinik für Kleintiere, Innere Medizin,
Justus-Liebig-Universität, Gießen
Betreuer: Prof. Dr. R. Neiger

24 hour hormone and serum electrolyte levels of dogs with pituitary-dependent hyperadrenocorticism treated with trilostane

INAUGURAL-DISSERTATION
zur Erlangung des Grades eines
Dr. med. vet.
beim Fachbereich Veterinärmedizin
der Justus-Liebig-Universität Gießen

eingereicht von

Claudia Lehnert
Tierärztin aus Leverkusen

Gießen 2007

Mit Genehmigung des Fachbereichs Veterinärmedizin
der Justus-Liebig-Universität Gießen

Dekan:	Prof. Dr. Reinacher
Gutachter:	Prof. Dr. R. Neiger
	Prof. Dr. R. Gerstberger

Tag der Disputation: 15.01.2007

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mit durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" niedergelegt sind, eingehalten.

Claudia Lehnert
Bergisch Gladbach 2007

Table of Contents

I. Introduction

Hyperadrenocorticism (HAC) in dogs is caused either by a cortisol secreting adrenal tumour or a pituitary adenoma secreting high doses of adrenocorticotropic hormone (ACTH) which then stimulates the adrenal glands to produce high amounts of cortisol. The chronic excess of systemic cortisol results in multiple clinical signs of various organ systems.

Numerous medical and surgical treatments have been established. Adrenalectomy is the treatment of choice for adrenal tumours. Hypophysectomy is the best treatment option for pituitary-dependent HAC, however, this technique is not widely available. Thus, the emphasis in managing pituitary-dependent HAC is on pharmacollogical treatment. The most commonly used drug is mitotane, but various others have been tried, including ketoconazole, L-deprenyl and aminoglutethimide. However, because of variable efficacy and side effects none of them is entirely satisfactory.

Since the late 1990ies trilostane, a competitive and reversible inhibitor of the 3ß-hydroxysteroid dehydrogenase, has been used for the treatment of canine HAC. So far, the experiences with trilostane are promising. Unfortunately, no pharmacological data in dogs with HAC are available and possible alterations in serum electrolyte concentrations as well as steroid hormone levels throughout the day are still unknown.

This study investigated the use of trilostane for the treatment of canine pituitary-dependent HAC and its effects on serum sodium, potassium and calcium, endogenous ACTH, cortisol, renin and aldosterone. The serum concentrations of trilostane and ketotrilostane were also determined. Blood samples were collected over 24 hours once HAC had been satisfactorily controlled.

II. Literature Review

1. Hyperadrenocorticism in Dogs

Next to hypothyroidism, HAC is the most common endocrine disorder in dogs (47).

Spontaneous HAC is most frequently caused by an ACTH-secreting pituitary tumour. This form is usually termed pituitary-dependent hyperadrenocorticism (PDH). Only about 15 to 20% of dogs with spontaneous HAC have an adrenocortical tumour (AT) which can equally be an adenoma or adenocarcinoma (47).

Most symptoms of HAC are not the direct results of the tumour, although the expansion of a pituitary macroadenoma may cause seizures and other signs of the central nervous system; local or distant metastases of an adrenal adenocarcinoma may cause further unspecific clinical signs. More often, however, an increase of circulating glucocorticoids affecting multiple organ systems is the main reason for various signs of illness. Since these symptoms are not the subject of this study, for detailed information see Feldman and Nelson (2005) (17).

Once signalement, history and clinical examination lead to the suspicion of HAC, blood and urine should be analyzed. Typical results are a stress leucogram (neutrophilia, lymphopaenia, monocytosis and eosinophilia), high serum cholesterol and asymmetrically high values of alkaline phosphatase with moderate increases of other hepatic enzymes. The urinalysis may show isosthenuria or hyposthenuria. Signs of complications, such as glucosuria in connection with hyperglycaemia or a lower urinary tract infection may also be seen (48). Proteinuria is rather common in dogs with HAC even if signs of lower urinary tract infection are missing (29).

To ultimately confirm the diagnosis of HAC, more specific tests are necessary. The ACTH stimulation test, the low dose dexamethasone suppression test and the urine corticoid/creatinine-ratio are routinely used screening tests. The ACTH stimulation test demonstrates the reaction of cortisol release after administration of supraphysiological doses of ACTH. The low dose dexamethasone suppression test, on the other hand, demonstrates the suppression of cortisol levels after application of dexamethasone, which inhibits by feed-back mechanism the release of endogenous ACTH. Both tests have in common that they are acceptably sensitive but less specific. Because of its high sensitivity and its low specificity, a negative result of the urine corticoid/creatinine-ratio can quite securely rule out HAC, but it is not qualified to make the diagnosis (33). Unfortunately, dogs with severe non-adrenal diseases may show positive test results in all three screening tests due to chronic stimulation of the hypothalamus-hypophysis-adrenal gland axis (33).

Several possibilities exist to differentiate between PDH and AT, the two best being to analyse both adrenal glands ultrasonographically (3,24) or to measure plasma endogenous ACTH (61,72). Because of these newer possibilities, the high dose dexamethasone suppression test is mainly indicated if neither the possibility to perform ultrasound nor to measure endogenous ACTH is given (47).

2. Treatment of hyperadrenocorticism in dogs

Microsurgical transsphenoidal hypophysectomy is an effective method of treating PDH (40) because it erases both the clinical signs caused by the excessive secretion of glucocorticoids and eventually occurring central nervous symptoms caused by the expansion of the tumour. Nevertheless, it requires considerable surgical expertise, represents a risky procedure and is not widely available. While radiotherapy reduces the size of pituitary tumours, it does not result in adequate control of clinical signs of HAC (21). Therefore, most dogs with PDH are still treated pharmacollogically.
In contrast, hormone-producing adrenal masses may be surgically removed. Nevertheless, some owners decide against surgery mainly because of the advanced age of their dog or due to the risk and the financial constraints.

Mitotane (o,p'-DDD) is the drug most commonly used for the treatment of HAC. It causes progressive necrosis of the adrenal cortex. Although it selectively destroys the glucocorticoid-producing zona fasciculata, the production of mineralcorticoids from the zona glomerulosa may become affected as well, with the risk of developing hypoadrenocorticism. In a study including 200 dogs with PDH treated with mitotane (55), approximately 30% of the dogs showed adverse effects such as lethargy, anorexia, neurological dysfunction, vomiting or diarrhoea. Eleven of the patients developed signs of glucocorticoid/mineralcorticoid deficiency and 2 died as a result of an Addisonian crisis. In 53.5% of the dogs, relapses were common at least once in the course of therapy. In addition, its adrenolytic effect is potentially harmful to the owners who handle the drug regularly. Mitotane can be used to treat both AT as well as PDH.

Several other medical treatments for HAC have been tried, all with variable success, side effects and cost.

Ketoconazole, an imidazole derivative commonly given as an antifungal agent, inhibits cytochrome P450-dependent enzymes. It specifically reduces serum cortisol levels whilst sparing the aldosterone synthesis (36). 20% to 25% of the dogs in one study did not respond to ketoconazole (15). The hepatotoxic potential also limits its use.

Another drug tried to treat canine HAC is selegeline hydrochloride or l-deprenyl. Regularly used to treat Parkinson's disease in humans, it represents a selective and irreversible inhibitor of monoamine oxidase type B. Thus, serum concentration of dopamine increases which causes a decrease of endogenous ACTH and thereby a decrease of serum cortisol. Although the drug is safe for pet and owner, only 10% to 20% of the dogs treated showed clinical improvement (7,59). This is not unexpected since selegeline mainly has an effect on the intermediate part of the pituitary gland while canine PDH mainly derives from the pars distalis.

In 2002 aminoglutethimide was evaluated as an alternative therapy to mitotane. It inhibits the conversion of cholesterol to pregnenolone in the adrenal cortex, thereby suppressing steroidogenesis. In high doses, it inhibits 11ß-hydroxylase and reduces cortisol, aldosterone and adrenal sex hormone production (56). Perez et al. (56)

found it to be inefficient at reducing cortisol levels. It also showed marked hepatotoxicity and can therefore not be recommended.

2a. Trilostane

Pharmacology

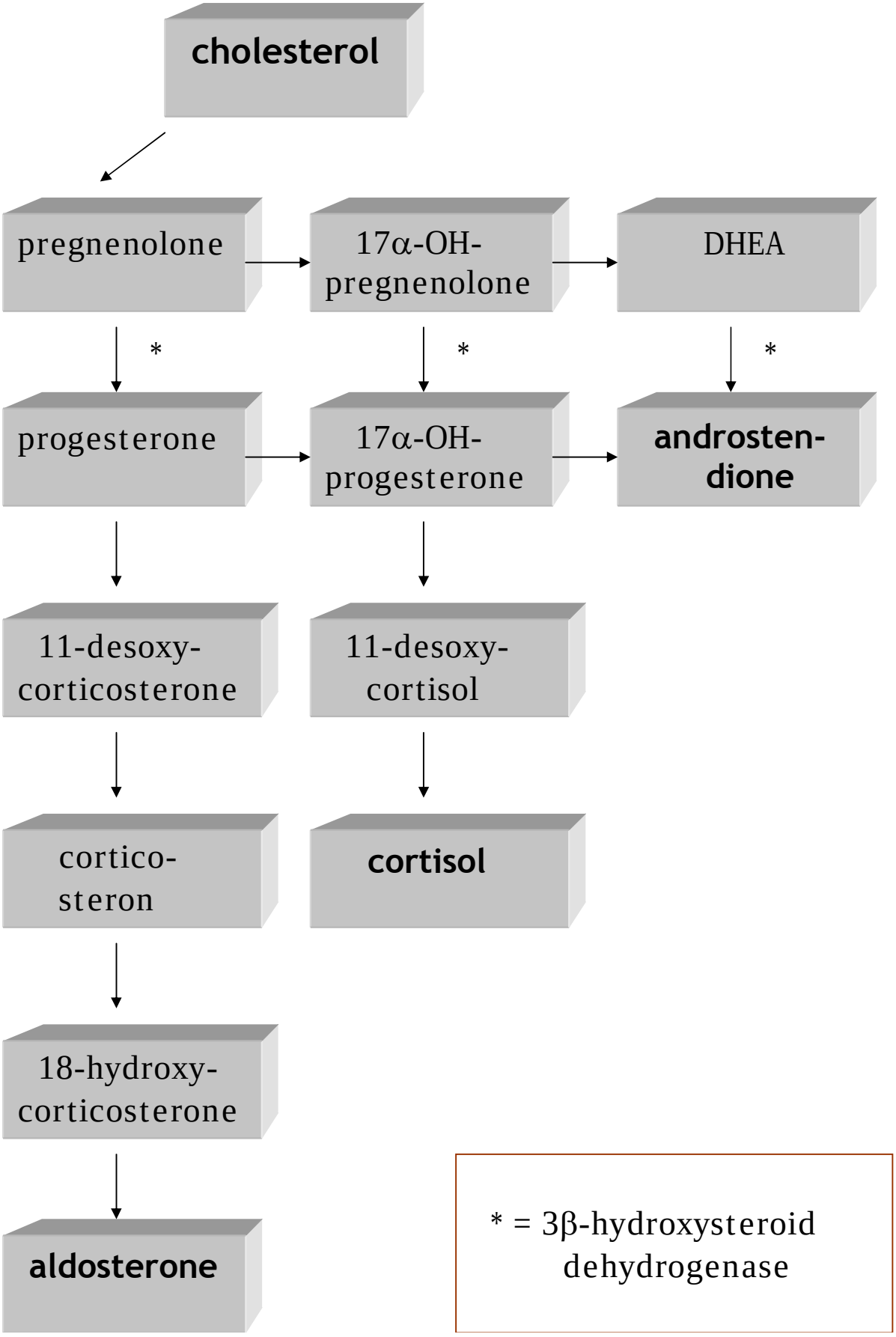
Trilostane is a synthetic, orally active steroid analogue. It acts as a competitive inhibitor of the 3ß-hydroxysteroid dehydrogenase enzyme system and thereby inhibits the synthesis of several steroid hormones, including cortisol and aldosterone. This blockage is reversible and seems to be dose-related (57). Because of this block, an accumulation of steroid hormone precursors above the site of the enzymatic inhibition can be found (66).

Pharmacologic data on trilostane uptake and release are available on rats (1,57,39), monkeys (1) and humans (60) and show quite marked differences amongst these species. In healthy beagle dogs trilostane is rapidly absorbed after oral application with peak blood concentrations found within 1.5 hours. Trilostane plasma levels decrease to baseline values in about 18 hours. There is quite a variation of plasma trilostane levels in these dogs, possibly in part due to the low water solubility of trilostane and thus erratic absorption from the gastrointestinal tract (data on file – Arnolds Ltd).

In vivo, trilostane and its metabolite ketotrilostane seem to be present in a state of equilibrium. Once trilostane is given orally, ketotrilostane develops rapidly. Correspondingly, if ketotrilostane is given to rats, trilostane is formed within a few minutes (39). Ketotrilostane shows 1.7-times the activity of trilostane in the inhibition of steroidogenesis (60).

After metabolism of trilostane into one of 4 additional metabolites, trilostane and ketotrilostane are either excreted mainly via faeces, as found in rats, or mainly via urine, as found in monkeys (1). So far the excretory pathway in dogs is unknown.

Figure 1: Major biosynthetic pathways of adrenocortical steroid biosynthesis.
The final end products are in bold. The asterix indicates the enzyme 3ß-hydroxysteroid dehydrogenase wich is blocked by trilostane.



Use in humans

Trilostane was originally developed for human medicine. Although most cases of HAC in humans are treated surgically, several studies have examined the use of trilostane in the treatment of inoperable or already metastasized tumours. Indications to use trilostane in humans are incomplete remission after surgery or the need of a symptomatic therapy before surgery in patients in serious conditions like hypertension or diabetes mellitus (62).

First experiences of 6 patients with HAC treated with trilostane were published by Komanicky et al. in 1978 (34). They concluded that trilostane was an effective drug with minimal side effects and satisfactory improvement in clinical and biochemical parameters. However, more recent work has cast some doubt on these conclusions. While in one study, half of the patients responded to therapy (65), in another study the 7 patients treated with trilostane showed neither a consistent fall of serum cortisol levels nor a rise in steroid precursors (12). These observations suggest that trilostane is not useful in the treatment of human HAC.

Whilst the results in treating human HAC are not satisfying, various studies show that it is useful in the treatment of both primary (43,50,72) and secondary hyperaldosteronism (23).

Imbalances of sex hormones, especially a postmenopausal increase of oestrone, are discussed to trigger human breast cancer (53). Since trilostane also shows some effect on the biosynthesis of sex hormones, it may be used in chemotherapy protocols of postmenopausal breast cancer. Beardwell et al. (4) showed that androstendione, 17-hydroxyprogesterone, pro-gesterone, testosterone and oestradiol decrease during trilostane therapy while oestrone increases. Several studies showed some success of trilostane in breast cancer therapy (10,30,72). The outcome was best after previous hormonal therapy (30). Chu et al. found a complete remission in 4% and a partial response in 19% of the patients (10). In contrast, Coombs et al. concluded that trilostane is not useful in human breast cancer. Gaining information from 41 patients with advanced breast cancer, only one responded to therapy and in six patients the condition stabilized with the remaining failing to respond (11).

Regardless of the reason for trilostane treatment, mostly gastrointestinal adverse effect were noted, ranging from slight, self-limiting diarrhoea (23,73), abdominal discomfort, light-headedness (30), facial flushing, oral paraesthesia and nausea (10) to fierce diarrhoea, abdominal cramping (10) and leucopaenia (30). One case report deals with an Addisonian crisis caused by trilostane (70). Because of its effect on male sex hormone biosyntheses, Semple et al. point to the possibility of sexual dysfunction during trilostane therapy (65).

Use in canine pituitary-dependent hyperadrenocorticism

The first positive experiences with trilostane in dogs with PDH were published in abstract format in 1998 (28). Since then, there have been several controlled studies (5,41,45,61) showing that trilostane can be used effectively and safely for the treatment of canine PDH.

Published in 2002 (45), a study of 78 dogs with PDH arrived at the conclusion that trilostane was effective and well tolerated by almost all dogs. Only two dogs showed signs of hypoadrenocorticism and two dogs died of unknown cause shortly after starting treatment. There was a significant reduction of both the mean basal and post-ACTH cortisol concentrations after a mean of 12.3 days of treatment. Polyuria and polydipsia resolved in 70% of the dogs presented with these problems and the skin of 62% of the dogs with dermatological changes normalized due to therapy. The dogs were treated and documented for up to three years.

In the same year data on clinical improvement and laboratory testing of 11 dogs with PDH treated with trilostane showed similar results (61). In addition, changes in the ultrasonographic appearance of the adrenal glands were noted in the course of therapy (37). This confirmed what Ruckstuhl et al. had described earlier (61). They found that trilostane causes enlargement of the adrenal glands with a maximum size achieved at about 6 weeks after initiation of treatment and minor fluctuations of size thereafter. Furthermore, there were noticeable changes within the parenchyma: The differentiation between the two layers of the glands (cortex and medulla) became more apparent, attributable to an increase of the hypoechoic outer zone and a decrease in the hyperechoic centre. After long-time treatment, the shape of the adrenal glands was irregular and the parenchyma had a heterogeneous echogenity. It appears that these changes reflect nodular hyperplasia as a morphologic consequence of long-standing hypersecretion of ACTH. The authors state that it cannot be ruled out that these changes may represent some kind of pre-cancerosis.

Successful treatment in 29 of 30 dogs with PDH was also reported by Braddock et al. (5).

A further study of 60 dogs (42 dogs with PDH, 11 with AT and 7 unclassified) investigated mainly satisfaction of owners and improvement of clinical and laboratory parameters over a period of 6 months (41). It found significant decreases in serum cholesterol, serum alkaline phosphatase and alanine transaminase levels. However, 28 of the 60 patients still had alkaline phosphatase levels above the reference range at the end of the study. Furthermore, there was a significant increase of serum potassium while the serum sodium levels decreased significantly. On 24 occasions, a sodium-potassium-ratio of less than 24 was found. However, hyperkalaemia was never treated in any of these incidences. While mild, self-limiting side effects such as diarrhoea, vomiting and lethargy were noted by 63% of owners mainly at the beginning of therapy, overall improvement of clinical signs was seen in 83% of the patients.

Including all 4 studies mentioned above, the mean starting dose was about 6 mg/kg once daily (5,41,45,61). More than 50% of the patients needed a dose adjustment, mainly a dose increase. Most dogs were initially quite sensitive to the drug for 10 to 30 days and then required a higher dose. This resulted in a final dose of 6.1 to 11.4 mg/kg in three of the studies (41,45,61). Since Braddock et al. (5) aimed at a markedly lower post-ACTH cortisol concentration, there was a much higher mean final dose of 18.1 mg/kg documented. The highest dose given to a dog was 48.7 mg/kg without side effects. Some of the dogs treated for more than 2 years required

reduction or temporary cessation of trilostane; the reason why these animals became more sensitive is unknown (5).

In a recent study performed by Sieber-Ruckstuhl et al. (66) several hormones such as cortisol, aldosterone, androgen, endogenous ACTH and steroid precursors both located before and after the 3ß-hydroxysteroid dehydrogenase were measured before and at two time points after starting treatment with trilostane. Some of the results were as expected, but others did differ from what was expected. Most important was that levels of steroid precursors localised below the 3ß-steroid dehydrogenase enzyme system were unchanged or even increased. This suggests strongly that trilostane inhibits not only the 3ß-steroid dehydrogenase – as already known (57) – but also other enzymes, most likely the 11ß-hydroxylase and possibly the 11ß-hydroxysteroid dehydrogenase (66). Further studies have to show whether these interpretations are true.

Hypoadrenocorticoid signs

In human beings, one case of an Addisonian crisis under trilostane therapy was described (70).

In the four studies mentioned above (5,41,45,61), 180 dogs with PDH were treated with trilostane. Only 2 dogs showed signs of hypoadrenocorticism. None of them died because of an Addisonian crisis, but two dogs participating in one study died of unknown cause (45). Melville-Walker et al. (41) found 24 occasions with sodium/potassium-ratios of less than 24, but none of the patients showed signs of hypoadrenocorticism. Eastwood et al. (14) describe five cases of prolonged hypoadrenocorticism under trilostane treatment. The patients showed extreme lethargy, weakness and poor appetite; low basal cortisol concentrations and a lack of response to exogenous ACTH were documented. Hypoadrenocorticism lasted up to 4 months. Where the patients did not fully recover, trilostane induced adrenal necrosis was considered possible.

Chapman et al. (8) observed clinical and biochemical changes suggestive of hypoadrenocorticism in a dog shortly after beginning therapy with trilostane. A histopathological analysis after exploratory laparotomy and excisional biopsy revealed adrenal cortical necrosis with reactive inflammation and fibrosis. It was hypothesed that, after losing its negative feedback mechanism, the resulting elevation of endogenous ACTH may cause adrenal necrosis as formerly described in man.

Sieber-Ruckstuhl et al. (66) realized that, although low aldosterone levels were measured after mitotane and after trilostane treatment. Dogs which were given mitotane regularly showed signs of aldosterone deficiency while dogs on trilostane treatment still appeared healthy. The authors developed the theory that steroid precursors accumulating due to the blockade of 3ß-hydroxysteroid dehydrogenase and supposedly other enzymes may also have cortisol effect.

Wenger et al. (25) also noticed that there seems to be no correlation between serum concentrations of aldosterone and potassium during treatment. Thus, there has to be at least one other mechanism that regulates the plasma potassium concentration. A direct effect of trilostane is possible. In contrast to this thesis, Potts et al. (57) could not verify any direct or indirect hormonal activity of trilostane in rats.

Use in canine adrenal-dependent hyperadrenocorticism

Trilostane has been under veterinary product licence only for the treatment of canine PDH and recently was also approved for the treatment of AT. Although 15 to 20% of the cases of canine spontaneous HAC are adrenal-dependent (47), the number of cases described involving a cortisol secreting adrenal mass is limited. One of the reasons may be that the treatment of choice for AT is the surgical excision. Two of the studies mentioned above also dealt with the trilostane treatment of AT (41,61), describing a clinical outcome which was comparable to the results of the treatment of the dogs with PDH. In 2003, a case report about the successful treatment of a dog with adrenal-dependent hyperadrenocorticism was published by Eastwood et al. (15). The mean survival time of AT depends on the malignancy of the tumour whose tendency to metastasize is not influenced by trilostane. Therefore, trilostane may be inferior to mitotane for the prevention and control of metastatic disease (45).

3. Effect of trilostane on hormones and electrolytes

3a. Physiology

Cortisol

In dogs, cortisol is the most important endogenous glucocorticoid hormone. It increases gluconeogenesis and thus increases glucose levels through the metabolism of amino acids. While parts of these glucose molecules are stored in the liver, glucose is also released into the blood stream. Cortisol has also an antagonistic effect on insulin which contributes to this increase of blood glucose since glucose uptake into cells is decreased. Because the amino acids are transformed into carbohydrates, cortisol has a catabolic effect. Muscle weakness and general lethargy are the results. Cortisol is also lipolytic with high plasma levels of fatty acids and lipoproteins. This results in a redistribution of body fat (47).

Cortisol causes immunosuppression, for example through inhibiting the effect of lymphocytes, via reducing lymphocyte cell division and antibody release (27). Polyuria and polydipsia are frequently found in patients with increased cortisol levels. Cortisol has an antagonistic effect on the antidiuretic hormone receptors in the collecting ducts of the kidney. Thus, the urine concentrating ability is markedly reduced. Furthermore cortisol prolongs the telogenic state of hair follicles, thus lost hair growth is reduced.

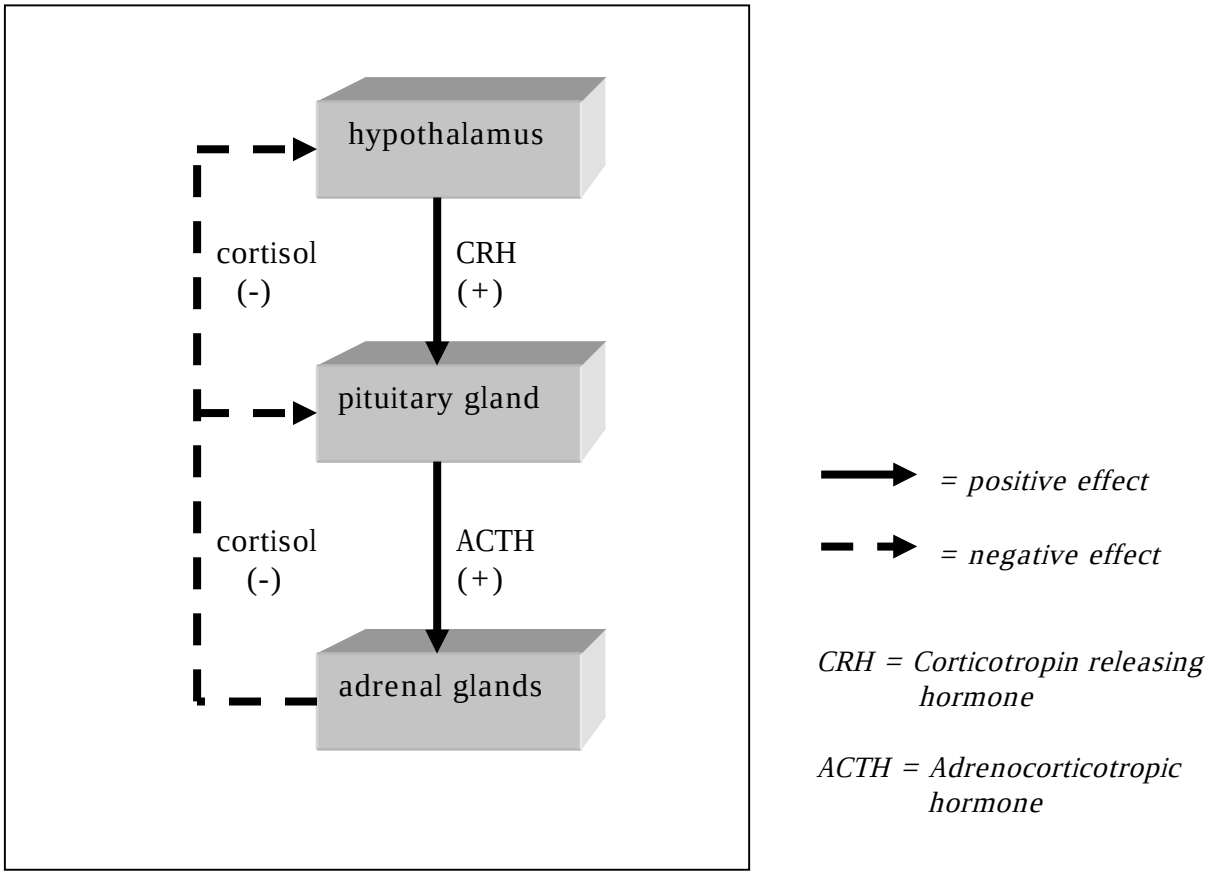
Endogenous ACTH

The secretion of ACTH is regulated by corticotropin-releasing factor (CRF), a peptide produced and secreted in the paraventricular nucleus of the hypothalamus. This nucleus in turn receives many nervous connections from the limbic system and lower brain stem. Initiated by physical or mental stress CRF is produced and secreted and it influences the release of ACTH from the anterior pituitary gland. The anterior pituitary gland can secrete only minute quantities of ACTH in the absence of CRF. ACTH stimulates the secretion of cortisol from the adrenal glands, but it also enhances the production of adrenal androgens. Through a negative feedback mechanism, high amounts of cortisol in the blood stream inhibit further secretion of ACTH by inhibiting the hypothalamus to produce CRF and at the same time inhibit the pituitary gland to produce ACTH (25).

ACTH partially influences plasma aldosterone levels. Small amounts of ACTH are required for aldosterone secretion, providing a permissive role that allows the other, more important factors like potassium and angiotensin to exert their more powerful controls (25).

Endogenous ACTH is increased or decreased, depending on the cause of HAC. In PDH excessive ACTH from an autonomic producing tumour stimulates both adrenal glands to produce excessive amounts of cortisol. As a result the adrenal glands become hyperplastic. In contrast, a cortisol-producing tumour of one adrenal gland leads to atrophy of the contralateral organ since the negative feedback mechanism results in almost absent plasma ACTH concentration (25,47).

Figure 2:
The hypothalamus-pituitary gland-adrenal gland axis.
CRH (Corticotropin releasing hormone) leads to the release of ACTH (adrenocorticotropic hormone) which causes the release of cortisol. High cortisol levels have a negative feedback on CRH and ACTH release (27).



Renin and aldosterone

Renin is an enzyme produced from prorenin in the cells of the glomerular afferent arterioles of the kidneys. These cells act as miniature pressure transducers that sense renal perfusion pressure. The most important stimulus for the release of renin comes from the sympathetic, ß1-adrenergic innervation of the cells of the glomerular afferent arterioles. The release of renin is also controlled by humoral factors such as

potassium, angiotensin II and atrial natriuretic peptide. Renin catalyses the formation of the decapeptide angiotensin I from angiotensiogen; angiotensin I is further converted through angiotensin converting enzyme into the octapeptide angiotensin II. Angiotensin II has direct vasoconstrictory effects and induces the secretion of aldosterone (25).
The plasma renin level is an accepted index to assess the renin-angiotensin system because it is difficult to measure other components (for example angiotensin II) because they are not stabile and diagnostic tests are not commonly available (32).

Aldosterone is a potent mineralcorticoid with two important actions: it regulates extracellular fluid volume and it is a major determinant of potassium homeostasis. Aldosterone acts predominantly on the distal convoluted tubule where it increases the reabsorption of sodium and the excretion of potassium.
Four mechanisms are known to influence the secretion of aldosterone (25): The potassium concentration and the renin-angiotensin-aldosterone-system play the greatest role; serum sodium levels and ACTH-secretion have minor influences. A very low percentage change in potassium concentration can cause a several-fold change in aldosterone secretion. Thus, aldosterone activates the secretion of potassium and the reabsorption of sodium.
The renin-angiotensin-aldosterone-system is activated in response to diminished blood flow to the kidneys as well as other stimuli (25).

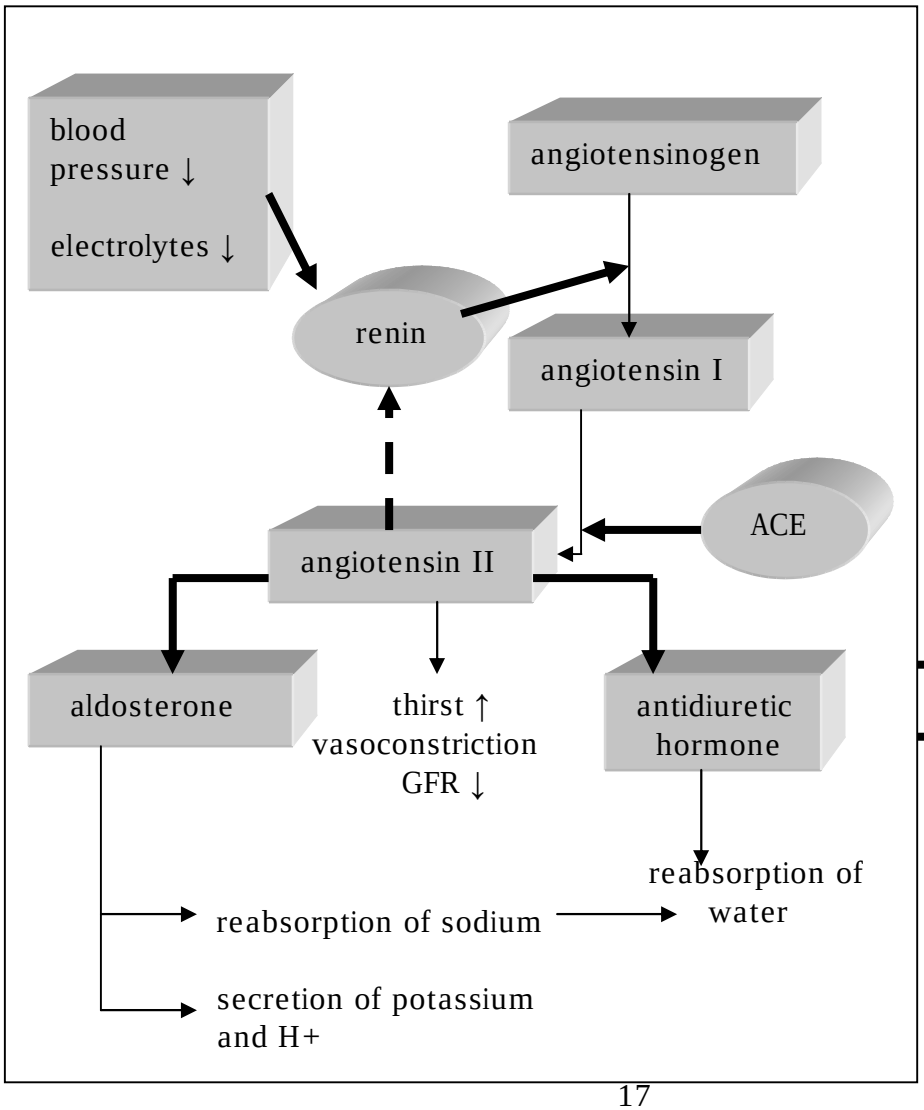


Figure 3:
Regulation and effects of the renin-angiotensin-aldosterone-system (27).

ACE = angiotensin converting enzyme

GFR = glomerular filtration rate

→ = positive effect

⊣ = negative effect

↑ = increase

↓ = decrease

Javadi et al. (32) have measured plasma aldosterone concentration and plasma renin activity in healthy dogs and in dogs with untreated HAC. Surprisingly, they found that chronically increased ACTH levels lead to a marked reduction of aldosterone levels. A decrease in aldosterone was already described by Golden et al. (20) in 1988; in addition, after stimulation with ACTH, they found increased levels similar to the concentrations of healthy dogs. Basal aldosterone levels are significantly lower in dogs with PDH than in healthy dogs; basal aldosterone concentrations in dogs with AT are significantly higher than in dogs with PDH (32). The authors speculate that persistent ACTH excess may cause aldosterone-secreting cells to change into cortisol-secreting cells. After administration of dexamethasone (0.1mg/kg), aldosterone concentrations decreased significantly to about 60% of the initial values in healthy dogs but no change is seen in dogs with PDH or AT.
ACTH has a direct role in regulating aldosterone release under physiological conditions. This seems not to be true in dogs with PDH, because the dexamethasone-induced decrease in plasma ACTH concentrations was not associated with any change in aldosterone concentrations (32).

The plasma levels of renin are similar in healthy dogs, dogs with PDH and dogs with AT (32). In dogs with PDH, renin activity increases significantly after administration of dexamethasone (0.1mg/kg). In dogs with AT, on the other hand, there is a noticeable, albeit non-significant increase of renin after dexamethasone (32). There are no differences in sodium and potassium levels between healthy dogs and dogs with HAC (32). The authors conclude that dexamethasone has two counteracting effects in dogs with PDH. On one hand it causes an increase in renin activity, while on the other hand it leads to a moderate decrease in circulating ACTH levels. Thus, the aldosterone concentration remains unchanged (32).

Serum electrolytes

Serum sodium and potassium levels are mainly regulated by the renin-angiotensin-aldosterone system. Sodium is the major electrolyte of the extracellular compartment. Aldosterone causes reabsorption of sodium in the distal tubule of the kidneys (27). Amongst other variables such as urea, the permeability for sodium, chloride and water in the collecting tubules is regulated by the osmolal gradient of the kidneys and by anti-diuretic hormone. In contrast to aldosterone, atrial natriuretic peptide causes an increase of sodium secretion in the kidneys. The pressure inside the right atrium regulates its release (27).
Potassium is the major intracellular electrolyte. Under aldosterone influence, potassium is actively secreted in the distal tubule (27).
Calcium levels are regulated by the complex hormonal influences of parathormon, calcitonin and vitamin D (1,25-dihydroxycholecalciferol). Aldosterone has no effect on serum calcium levels. It is well known that cortisol has calcium-lowering potential which is frequently used in the medical treatment of hypercalcaemia (47).

3b. Effect of trilostane

Cortisol

As stated previously, cortisol is markedly increased in both conditions: PDH and AT. After treatment with trilostane, cortisol is significantly reduced (5,45,61,66,71). This reduction is used to objectivate therapy success by measuring basal and post-ACTH cortisol levels.

Trilostane's inhibitory effect on cortisol concentration lasts about 12 hours in dogs with PDH, while the clinical effect seems to last longer (45). This is surprising since trilostane plasma levels in healthy dogs peak after 1,5 hours and decrease quite rapidly thereafter (Arnolds, data on-file). Nonetheless clinical experience dictates that in most cases trilostane application once daily is sufficient (5,45,61).

Treatment with mitotane results quite commonly in Addison's disease (55). Interestingly, despite basal and post ACTH cortisol concentrations below 25 nmol/l, clinical signs of adrenocortical insufficiency are very infrequently seen in dogs treated with trilostane (61). It is speculated that steroid precursors that have accumulated as a result of enzyme inhibition have certain glucocorticoid-like effects (66).

Endogenous ACTH

Endogenous ACTH plasma levels are significantly elevated in dogs with PDH following trilostane therapy compared to pre-treatment values (74). Furthermore, there is a negative correlation between the drug-dosing interval and the ACTH concentration, with higher ACTH levels seen in dogs which had received trilostane more recently (74). The authors suggest that concurrent reduction in serum cortisol concentrations may result in higher levels of endogenous ACTH, following the removal of the negative feedback mechanism of high cortisol levels. A recently published study by Sieber-Ruckstuhl et al. (66) has confirmed these findings.

Renin and aldosterone

In dogs with HAC undergoing treatment with trilostane, aldosterone is less influenced by trilostane than cortisol. Wenger et al. (71) have measured basal cortisol, basal aldosterone levels and cortisol and aldosterone concentrations after stimulation with ACTH. Samples were taken before and 1, 4, 8 and 12 weeks after initiation of trilostane treatment. No significant changes in basal aldosterone levels were found while serum cortisol was significantly more reduced than aldosterone after stimulation with ACTH. Only recently, a significant increase in basal aldosterone levels after trilostane therapy was found with decreased post-stimulation aldosterone levels (66).

Although there is data available on plasma renin activity in healthy dogs and dogs with HAC (32), no study has yet been done to evaluate the renin activity in dogs with HAC undergoing trilostane therapy.

Serum electrolytes

In two studies, serum sodium levels were measured. While 6 of 11 dogs were hypernatriaemic before the onset of therapy, only two dogs showed sodium levels above the normal range at the fifth re-evaluation (61). Potassium levels above the reference range were found in 6 of 11 dogs while none of them was hypokaleamic.

In another study (41), sodium levels decreased significantly under trilostane therapy. In this study, two dogs had sodium/potassium-ratios of less than 24 six months after starting therapy with trilostane. 8 of 60 dogs had potassium levels above the normal range six months after starting trilostane therapy.
Wenger et al. (71) found that median potassium concentrations increased slightly under trilostane therapy. They found no correlation between serum potassium and aldosterone levels.
So far, calcium levels have not been measured under trilostane influence.

IV. Hypotheses

- ✓ After trilostane application, ketotrilostane is formed rapidly. Both trilostane and ketotrilostane are present in a state of equilibrium and circulate in the blood only for 10 to 18 hours after application.

- ✓ Cortisol levels are reduced significantly by trilostane; the maximum effect is reached after 3-4 hours. Decreased cortisol is noticed longer than trilostane and ketotrilostane are measured in the blood stream.

- ✓ In comparison to the levels before the initiation of therapy, ACTH levels are significantly increased after therapy with trilostane. During the day, maximum ACTH levels are expected when cortisol levels are at their trough level which will be 3 to 4 hours after trilostane uptake.

- ✓ In doses routinely used for the treatment of canine HAC, trilostane does not decrease the aldosterone concentrations to levels that may cause symptoms of a live-threatening hypoadrenocortic crisis

- ✓ Because there are only mild changes in aldosterone concentrations, the renin concentration remains stable 24 hours after trilostane application

- ✓ Because aldosterone levels remain constant, administration of trilostane does not change sodium and potassium levels significantly. Aldosterone and cortisol have only minor influence on calcium levels. Thus, no significant change of calcium levels is expected.

III. Materials and Methods

1. Trial procedure

This study was a prospective investigation. Its objective was to ascertain the daily fluctuation of once daily trilostane therapy on serum electrolytes (sodium, potassium, calcium), endogenous ACTH, renin, aldosterone and cortisol. Furthermore, plasma trilostane and ketotrilostane values were measured to ascertain the uptake and to investigate the pharmacologic property of this compound.

Primary diagnosis

Ten dogs diagnosed with PDH were included in the study. They had to weigh more than 15kg and had to be healthy with the exception of PDH and symptoms caused by excessive endogenous cortisol production. Dogs with AT were excluded.

Dogs with clinical signs suggestive of HAC were further diagnosed with an ACTH stimulation test, and/or a low dose dexamethasone suppression test. To distinguish between PDH and AT an ultrasound of the adrenal glands (where possible) and measurement of endogenous ACTH were performed (22).
The ACTH stimulation test and low dose dexamethasone suppression test were performed as described (47). Briefly, blood samples for determination of cortisol levels before and 1 hour after either IM or IV injection of 0.25mg synthetic ACTH (*Synacten®,* Versatis) were collected. Cortisol concentration was measured using a chemiluminescence method at Biocontrol Laboratories, Mainz, Germany. A cortisol concentration > 550 mmol/l in the sample collected 1 hour after ACTH administration was considered suggestive of HAC. For the low dose dexamethasone suppression test, blood samples were collected before and 4 and 8 hours after IV injection of 0.01 mg/kg dexamethasone. A cortisol concentration of >38.6mmol/l in the sample collected 8 hours after dexamethasone administration was considered to reflect a lack of suppression and was suggestive of HAC. If the cortisol concentration at 4 hours after dexamethasone administration was <38.6mmol/l or less than 50% of the baseline cortisol concentration PDH was confirmed.
Endogenous ACTH was measured from pre-analytically stabilized blood (stabilizer tubes containing a mixture of two protease inhibitors) using a two-site-chemoluminometric assay at Alomed Laboratories (α), Radolfzell (63).
Included were patients with at least one positive screening test results (ACTH stimulation test, low dose dexamethasone suppression test) and a safe discrimination into pituitary-dependence.
Adrenal ultrasound was performed at the Small Animal Clinic, Justus-Liebig-University, Gießen, Germany. Although the whole abdomen was visualized, the major focus was on the adrenal glands whose minimal and maximal size was measured as described previously (3,24).

Before treatment with trilostane, a blood sample for haematology and biochemistry was taken. All haematological and biochemical tests were performed at the central laboratory of the Small Animal Clinic, Justus-Liebig-University, Gießen. If available, a urine sample obtained by cystocenthesis was examined (urine dipstick, sediment and bacteriological examination). Dogs with positive urine culture were treated with

amoxicilline and clavulanic acid (20-25 mg/kg twice daily) for 2-3 weeks unless bacteria were found to be resistant.

Control after 10 days of therapy

After ten days of treatment, the owners were asked about their dogs' well being, amount of drinking and eating, frequency and quantity of urination and changes in agility and skin or hair condition. Possible side effects were also questioned. The dogs were then re-evaluated clinically and an ACTH stimulation test was performed. Post-ACTH stimulation test cortisol levels were aimed at less than 250 nmol/l. If cortisol levels were above 250 nmol/l or if the dog was clinically not well controlled, the trilostane dose was increased by 60 mg. Levels below 50 nmol/l were considered too low and the trilostane dose was reduced. If the dog showed adverse effects (lethargy, diarrhoea or vomiting), the drug was withdrawn and re-instituted after a few days at half the previous dose. A re-examination after 10 days was again performed in those dogs in which a change in the trilostane dose was needed.

Control after 30 days of therapy

Twenty days after the final dose change, the dogs were hospitalised for 36 hours and one day prior to blood sampling, a central venous catheter was inserted either into a jugular vein or into a femoral vein. Trilostane was administered the next morning at 7 o'clock (range 6:30 to 7:30 a.m.) with food. During that day, blood samples were taken at the following time points: -30, 0, 15, 30, 60, 90, 120, 180, 240 minutes, 6, 8, 12, 16, 20 and 24 hours post trilostane administration. An ACTH stimulation test was performed one day later, 3 to 4 hours after trilostane administration in the morning.

Blood was taken from the central venous catheter. The first 10ml of blood were dismissed, then 1,2ml of blood were put into each of the following tubes: EDTA tubes were used for measurement of ACTH and renin, heparin tubes for serum electrolytes, trilostane and ketotrilostane and tubes without additive for cortisol and aldosterone. After taking blood, heparinized sodium chloride solution was given into the central venous catheter. Next, serum electrolytes were measured immediately from whole blood. In the meantime, the other blood samples were centrifuged and separated immediately. The samples were stored at -80°C. Samples were shipped in two instalments on dry ice to the laboratories with over-night courier. Renin, ACTH, cortisol and aldosterone were measured at Cambridge Specialist Laboratories (β), Wallsend, UK while trilostane and ketotrilostane were measured at HFL Ltd. (γ), Fordham, UK.

2. Treatment regiment

After a final diagnosis, written consent was obtained from the owners and the dogs were given the following dose of trilostane:

Weight (kg)	*starting dose*
< 20	60mg once daily
20 - 40	120mg once daily
> 40	180mg once daily

3. Laboratory evaluation

Trilostane and ketotrilostane

A liquid chromatographic-tandem mass spectrometric (LC-MS/MS) bioanalytical assay for the measurement of trilostane and ketotrilostane has been developed and validated over the concentration range 5 – 1000 ng/ml in canine plasma (38) Trilostane and ketotrilostane were extracted from plasma using a solid phase extraction procedure. Chromatography was carried out using a reversed phase analytical column with an isocratic mobile phase. The analytes and internal standard were ionised using the TurbolonSpray™ interface on a Sciex 3000 instrument operating in negative ion mode.

Trilostane and ketotrilostane are stable in canine plasma for at least 20 months. The assay was robust, sensitive, and highly specific. No significant matrix interference was observed.

Cortisol

To measure serum cortisol levels, a radioimmunoassay (RIA) was used. It has been validated over the concentration range 20 – 1656 nmol/l. Normal dogs show serum cortisol levels up to 250 nmol/l.

ACTH

An immunoradiometric assay (IRMA) was used to measure endogenous ACTH out of EDTA plasma. A sandwich assay with solid-phase bead system was performed. The test has been validated over the concentration range 5.0 – 600 pg/ml. The reference range for normal dogs lies between 20 and 80 pg/ml (22).

Aldosterone

For serum aldosterone measurement, a radioimmunoassay (RIA) was performed, with solid phase coated tube separation (coat-a-count aldosterone). The sensitivity lies at 20 pmol/l, the upper limit is 3300 pmol/l. Reference range in normal dogs is up to 960 pmol/l (54).

Renin

Renin was measured using a radioimmunoassay (RIA), again with solid-phase coated tube separation (gammacoat plasma renin). The assay has been validated over the range 0.08 – 5.0 ng/tube. Reference range in normal dogs ranges from 0.22 – 2.4 ng/ml/hr (54,68).

Serum electrolytes

Serum electrolytes were measured with an ion-selective electrode system (AVL, Roche), which has been used extensively in veterinary medicine. Reference ranges in normal dogs for sodium are 141 – 152 mmol/l, for potassium are 3,6 to 5,4 mmol/l and for ionized calcium are 1,2 and 1,45 mmol/l.

4. Data Analysis

Normality of the data was analysed by the Shapiro-Wilk test. Logarithmic transformation was performed of data which were not distributed normally. An analysis of variance (ANOVA) with repeated measures (time) was performed for

transformed and non-transformed data. To assess at which time point a statistical change in comparison with the initial value could be seen, a Dunnett-test was used. The Friedman's test was used for data which were not normally distributed even after logarithmic transformation. Here pair-wise comparison between time-points and baseline was done by Wilcoxon-Wilcox. For statistical analysis, data points below the detection limit of the assay were given a number of the arithmetic mean between the lower assay level and zero. A P-value of ≤ 0.05 was considered significant.

V. Results

1. Baseline results

Ten dogs with PDH fulfilled the inclusion criteria and could be recruited for this study (for patient data see table 1). The mean (± SD) age and weight of the dogs were 9.3 ± 0.67 years and 31.9 ± 6.4 kg, respectively. The starting dose of trilostane ranged from 60mg to 180mg once daily with a mean of 4.2 ± 0.55mg/kg. Since all dogs were well adjusted on the 10th day of therapy, no changes of the dose were necessary.

Table 1: Signalement of dogs included in this study with trilostane dose used for treatment of pituitary-dependent hyperadrenocorticism.

PATIENT	BREED	SEX	AGE (y)	WEIGHT (kg)	TRILOSTANE DOSE (mg)	TRILOSTANE DOSE (mg/kg)
Dog 1	Irish Setter	m	9	27	120	4.44
Dog 2	German Short Hair	(m)	9	40	180	4.50
Dog 3	Mixed breed	(f)	13	15,5	60	3.87
Dog 4	Münsterländer	f	9	28	120	4.29
Dog 5	Pointer	m	8	36	120	3.33
Dog 6	Dalmatian	(m)	10	27	120	4.44
Dog 7	Staffordshire Bullterrier	(f)	9	32	120	3.75
Dog 8	Collie	m	9	42	180	4.29
Dog 9	Golden Retriever	(f)	10	32	120	3.75
Dog 10	Beagle	(f)	11	23	120	5.22

Legend: m = male; f = female; () = neutered/spayed
Trilostane dose: all doses given once daily

The dogs were presented to a veterinarian because of varying symptoms. Nine of the ten dogs were presented due to polyuria, polydipsia and/or nocturia. The owners of four of the dogs described a general weakness and difficulty to walk. Four dogs showed focal alopecia and signs of superficial pyoderma. Two dogs were presented because of excessive panting. One dog's owner did not notice polyuria/polydipsia (dog 10); this owner was concerned because of his dog's immense appetite and increasing weight.
The haematology and biochemistry results of all dogs before and 30 days after the onset of therapy are shown in table 3.

2. Clinical Improvement

Control after 10 days of therapy

After 10 days of therapy (range: day 9 to day 12), all patients had clinically improved. According to the owners, all but one dog (dog 9) showed a noticeable decrease of thirst and the quantity and frequency of urination diminished. Panting improved in two of two dogs (dogs 6 and 10) and all but one (dog 9) seemed to be more agile and generally feeling better. None of the dogs showed adverse effects. The girth and weight did not decrease in any of the dogs and all were still polyphagic. The skin condition of the patients with skin or hair problems (dogs 1, 4, 5 and 9) had not improved visibly.

An ACTH stimulation test was performed on every dog on the tenth day of therapy, 3 to 4 hours after trilostane administration in the morning. All dogs showed a significant reduction in post-ACTH cortisol levels (table 2).

Control after 30 days of therapy

Second re-examination happened on the 30th day of therapy (range: day 28 to day 35). All dogs were hospitalized and blood samples were taken at fixed time points. All owners but one (dog 9) reported improvement in clinical well-being. Although skin conditions had only improved slightly, hair started to re-grow in dogs with dermatological problems. None of the patients showed adverse effects and all but one dog (dog 9) seemed more agile than before treatment.

Haematology and biochemistry results had generally improved although not all were within normal limits (table 3 + 4).

Cortisol values pre and post ACTH stimulation test remained similar to the 10 day re-evaluation (figure 4, table 2) and the trilostane dose was maintained the same in all dogs.

Table 2: Results of ACTH stimulation test before and after onset of therapy with trilostane.

Before the onset of therapy, not all of the ACTH stimulation tests showed positive results; in these cases, hyperadrenocorticism was confirmed by other diagnostic tests. After 10 and 30 days of therapy, all patients' ACTH stimulation tests showed improved results.

	BEFORE THERAPY		**10TH DAY OF THERAPY**		**30TH DAY OF THERAPY**	
	Cortisol 0 hr (nmol/l)	**Cortisol 1 hr (nmol/l)**	**Cortisol 0 hr (nmol/l)**	**Cortisol 1 hr (nmol/l)**	**Cortisol 0 hr (nmol/l)**	**Cortisol 1 hr (nmol/l)**
Dog 1	193.13	595.94	77.25	63.46	115.88	154.50
Dog 2	80.01	397.30*	46.90	68.80	27.59	85.53
Dog 3	148.99	474.55*	16.55	41.39	60.70	80.01
Dog 4	118.64	653.88	63.46	115.88	35.87	135.19
Dog 5	129.67	471.79*	60.70	104.84	24.83	46.90
Dog 6	184.85	488.34*	33.11	74.49	19.31	132.43
Dog 7	209.68	830.46	22.07	102.08	13.80	44.14
Dog 8	171.06	565.60	30.35	35.87	44.14	60.70
Dog 9	107.60	618.02	35.87	37.95	< 13.80	38.63
Dog 10	148.99	811.15	77.25	124.16	35.87	118.64
Mean ± SD	149.26 ±41.06	590.70 ±144.14	46.35° ±22.18	76.89° ±33.13	39.18° ±30.58	89.67° ±42.69

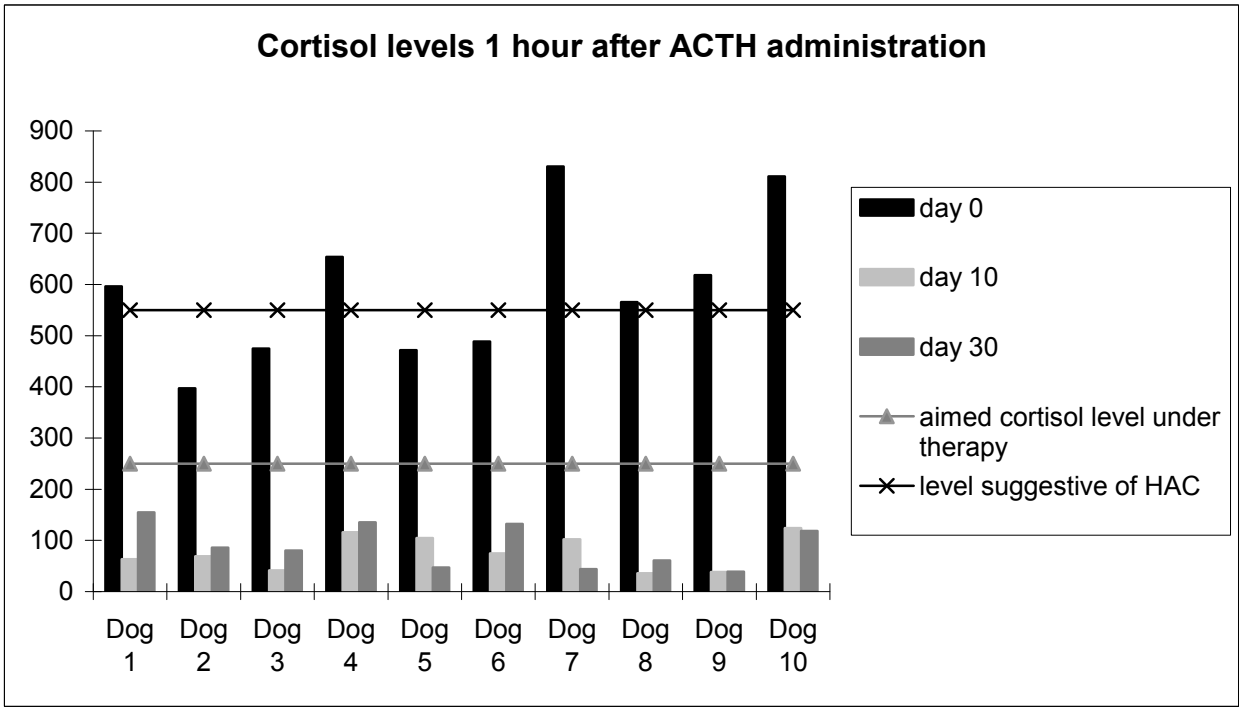
Legend:

** = ACTH stimulation test result not clearly positive; diagnosis was confirmed by low dose dexamethasone suppression test*

° = significant decrease to pre-treatment values

SD = standard deviation

Figure 4: Serum cortisol levels 1 hour after ACTH administration (day 0, 10 and 30 after start of trilostane treatment) in dogs treated with trilostane.
There was a significant reduction in both 10 and 30 days' cortisol levels with all dogs reaching the aimed level.



Legend:

ACTH = Adrenocorticotropic hormone
HAC = hyperadrenocorticism

Table 3: Blood haematology results before and 30 days after onset of trilostane therapy.

	NEUTRO-PHILS (10^9/L)		**LYMPHO-CYTES (10^9/L)**		**EOSINO-PHILS (10^9/L)**		**RED BLOOD CELLS (10^{12}/L)**		**PLATELETS (10^9/L)**	
	Day 0	**Day 30**	**Day 0**	**Day 30**	**Day 0**	**Day 30**	**Day 0**	**Day 30**	**Day 0**	**Day 30**
Dog 1	5.54	8.73	**0.67°**	2.90	0.07	0.75	5.96	5.98	**696***	**550***
Dog 2	4.17	6.09	**0.90°**	1.25	0.17	0.18	7.12	6.61	362	364
Dog 3	8.95	6.18	1.00	1.04	0.07	0.06	6.43	5.90	**678***	**530***
Dog 4	5.29	6.89	**0.74°**	1.46	0.07	0.28	8.06	7.72	433	333
Dog 5	6.56	8.96	1,35	1.56	0.09	0.21	7.00	7.74	445	217
Dog 6	10.03	6.51	**0.91°**	**0.55°**	0.00	0.12	7.8	**8.53***	**981***	**787***
Dog 7	10.83	5.28	**0.92°**	1.64	0.06	0.63	6.17	5.90	**634***	463
Dog 8	**12.1***	6.34	**0.97°**	1.08	0.02	0.16	7.21	7.52	465	239
Dog 9	9.97	8.23	**0.95°**	1.03	0.02	0.09	7.21	7.05	382	388
Dog 10	10.04	9.27	**0.87°**	1.11	0.16	0.13	8.14	7.98	494	399

Legend:

° = Results below the reference range
** = Results above the reference range*

Table 4: Some biochemistry parameters before and 30 days after onset of trilostane therapy.

ALP, ALT, GLDH and cholesterol levels have decreased significantly while serum osmolality levels only decreased slightly.

	ALP (U/l)		ALT (U/l)		GLDH (U/l)		Cholesterol (mmol/l)		Osmolality (mmol/kg)	
	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Dog 1	**2710***	**510***	**122***	25	**25.2***	1.0	6.1	4.38	**315***	**329***
Dog 2	**2171***	**570***	48	**74***	**12.9***	**19.1***	**10.0***	**7.7***	n.d.	n.d.
Dog 3	**593***	**514***	52	43	**15.7***	9.9	**9.6***	**7.1***	**332***	313
Dog 4	**1023***	**654***	**205***	**120***	**30.1***	**26.5***	6.9	5.7	n.d.	n.d.
Dog 5	**156***	**118***	33	32	**15.2***	9.6	**14.0***	**8.10***	**326***	312
Dog 6	**4470***	**3765***	**205***	39	**40.4***	**22.0***	6.6	**7.02***	n.d.	**325***
Dog 7	**3765***	**2240***	**151***	50	**39.0***	11.0	4.8	5.18	**325***	**317***
Dog 8	**1824***	**1700***	**112***	**113***	n.d.	**28.0***	**10.4***	6.76	n.d.	310
Dog 9	**1986***	**1535***	**158***	**141***	**17.0***	4.0	**7.1***	**8.11***	**364***	**285°**
Dog 10	**1265***	**958***	**116***	**68***	**17.9***	10.5	**9.6***	**7.18***	**334***	**315***
mean ± SD	1996.3 ±1360.3	1375.4 ±1072.9	120.2 ±61.7	70.5 ±40.8	23.7 ±10.5	14.2 ±9.2	8.5 ±2.7	6.7 ±1.3	332.7 ±16.7	313.3 ±13.2

Legend:

° = Results below the normal range
** = Results above the normal range*
n.d.= not done
ALP = alkaline phosphatase
ALT = alanine amino transferase
GLDH = glutamate dehydrogenase
SD = standard deviation

Complications

Dog 3 showed good clinical improvement upon therapy and was clinically well controlled after 10 and 30 days. Shortly before administration of trilostane during the hospitalisation period (day 31) the dog collapsed unexpectedly. After two minutes of unconsciousness, the animal was able to rise but was still depressed. Complete blood examination and thoracic radiographs did not reveal anything abnormal and the dog made an uneventful recovery. This dog was removed from the study as electrolyte and hormone parameters might be affected by this collapse.

Dog 9 still showed polyuria and polydipsia on its 10 and 30 day re-examination. However, the post-ACTH cortisol levels had normalised and complete blood examination revealed a decrease in ALP-levels. Furthermore serum osmolality was within normal limits indicating that there might be more likely primary polydipsia with compensatory polyuria. Additionally, the dog had great difficulty in raising or going up the stairs. It had a history of arthrosis in both knees and hips for many years and had undergone surgery because of a ruptured cruciate ligament unilaterally two years previously. Treatment with non-steroidal anti-inflammatory drugs was recommended and the dog showed improvement in its agility.

3. Plasma trilostane and ketotrilostane levels

In all but dog 7, maximal trilostane plasma levels were reached after a 1.5 to 3 hours. After reaching maximal concentration, mean trilostane plasma levels decreased rapidly and became undetectable 12 hours after trilostane administration. There was a significant increase of plasma trilostane levels between 1 and 4 hours post dosing ($P < 0.05$) (figure 5).
With the exception of dog 7, all dogs showed the same trilostane plasma curve, although the maximum concentration varied widely (dog 1: maximum levels of 2206 ng/ml; dog 5: maximum levels of 233 ng/ml).

Ketotrilostane plasma levels also peaked after 1.5 to 3 hours in all but one dog (dog 7). A significant increase of plasma ketotrilostane levels was seen between 1.5 and 4 hours post dosing ($p < 0.05$). After that, mean ketotrilostane plasma levels decreased rapidly (figure 6). Basically 12 hours after trilostane intake, ketotrilostane plasma levels were below the detection limit of the assay.

Figure 5: Mean serum trilostane levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.
The non parametric data were initially logarithmized and mean and standard deviation of these values were calculated. For graphic display these results were delogarithmized again. Positive whiskers indicate geometric mean times variance while negative whiskers show geometric mean devided by variance. Asterices show significant changes from postdosing levels.

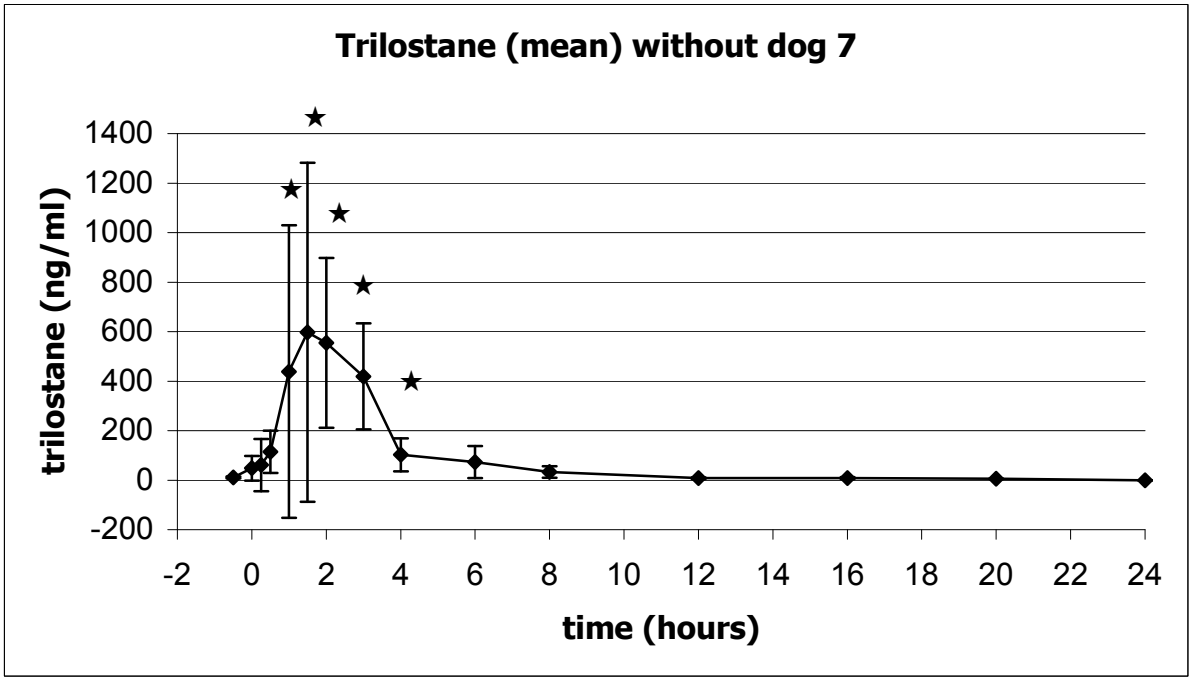


Figure 6: Mean serum ketotrilostane levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.

For explanation of graphical display see figure 5.

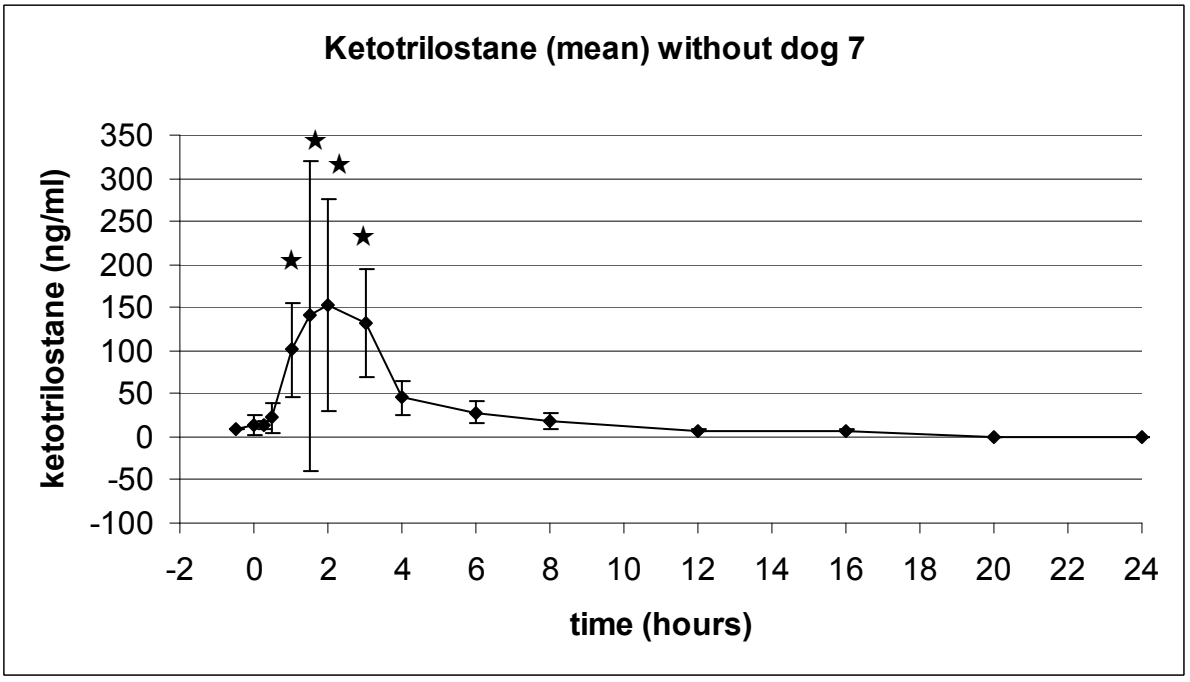
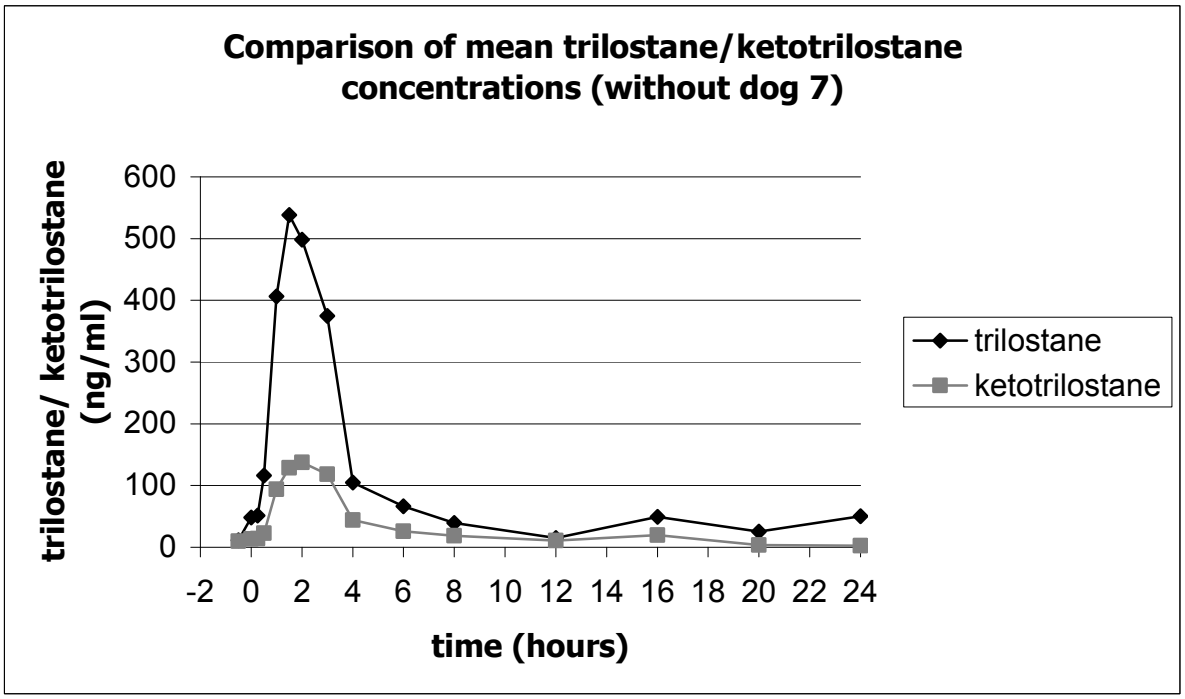
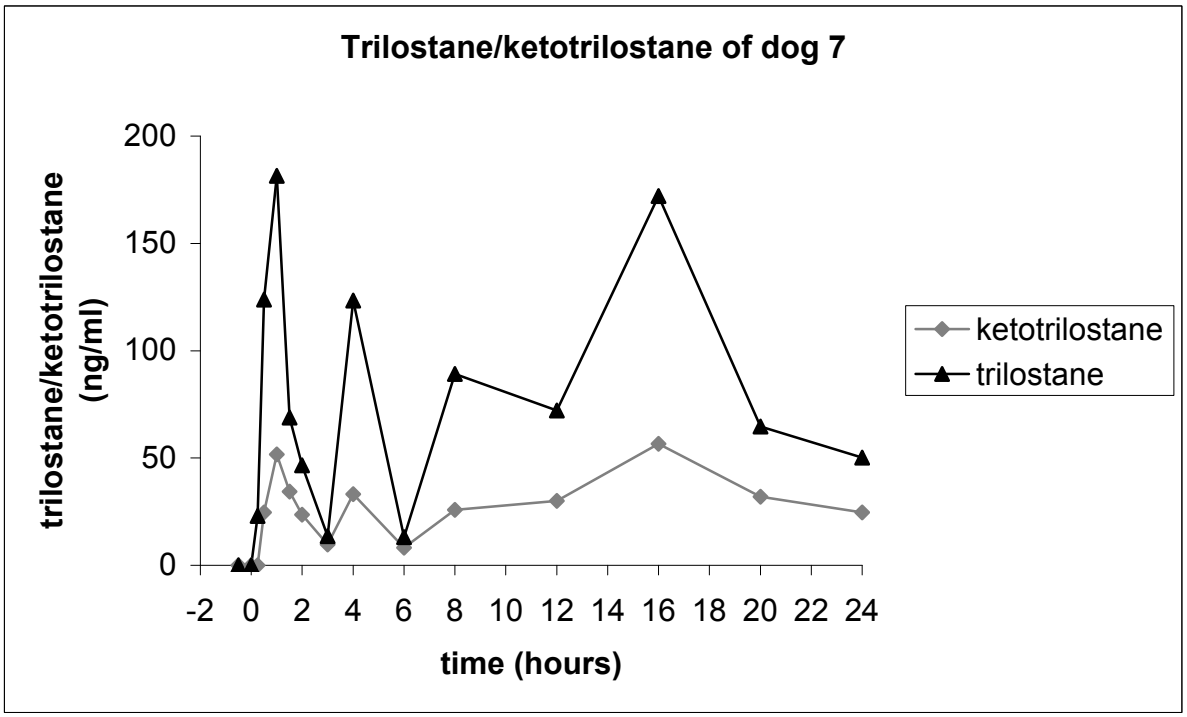


Figure 7: Mean trilostane and ketotrilostane levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.



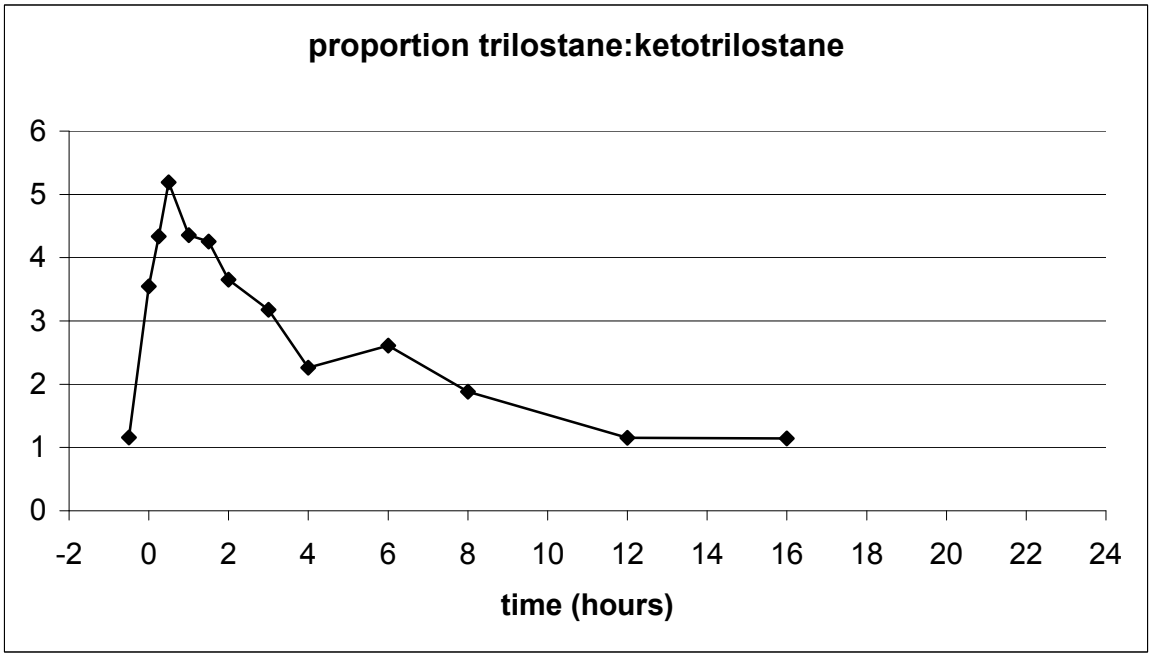
Trilostane and ketotrilostane plasma curves of dog 7 differ markedly from the other dogs (figure 8 and 9). While all the other dogs had one peak concentration, the plasma levels of trilostane and ketotrilostane peaked at 1, 4 and 16 hours after drug application. Interestingly, 30 minutes before trilostane application, which corresponds to 23½ hours after last day's trilostane application, neither trilostane nor ketotrilostane were detectable.

Figure 8: Serum trilostane and ketotrilostane levels of dog 7 on day 30 of treatment with trilostane.



The ratio of trilostane to ketotrilostane indicates that shortly after drug administration most trilostane is not yet converted to ketotrilostane, however over the next 16 hours, the ratio gradually decreases as more and more trilostane is converted to ketotrilostane (figure 9).

Figure 9: Ratio of serum trilostane to serum ketotrilostane in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane (all dogs except of dog 7).

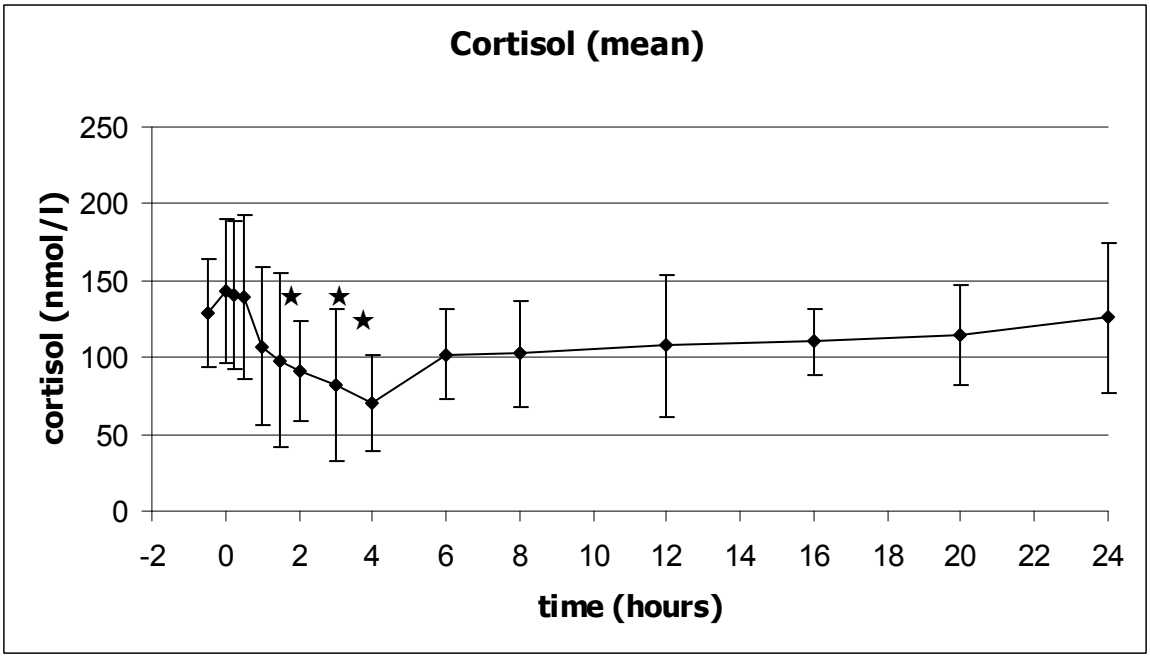


4. Cortisol

Mean serum cortisol levels decreased significantly after trilostane dosing ($P < 0.001$). Pair wise comparison between baseline value and time points showed a significant decrease between 2 and 4 hours ($P < 0.05$) post drug administration (figure 10). In six dogs, the lowest cortisol concentration was below the detection limit of the assay at least once during the day. No adverse clinical effects were seen in any of the dogs at those time points.

Figure 10: Mean serum cortisol levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.

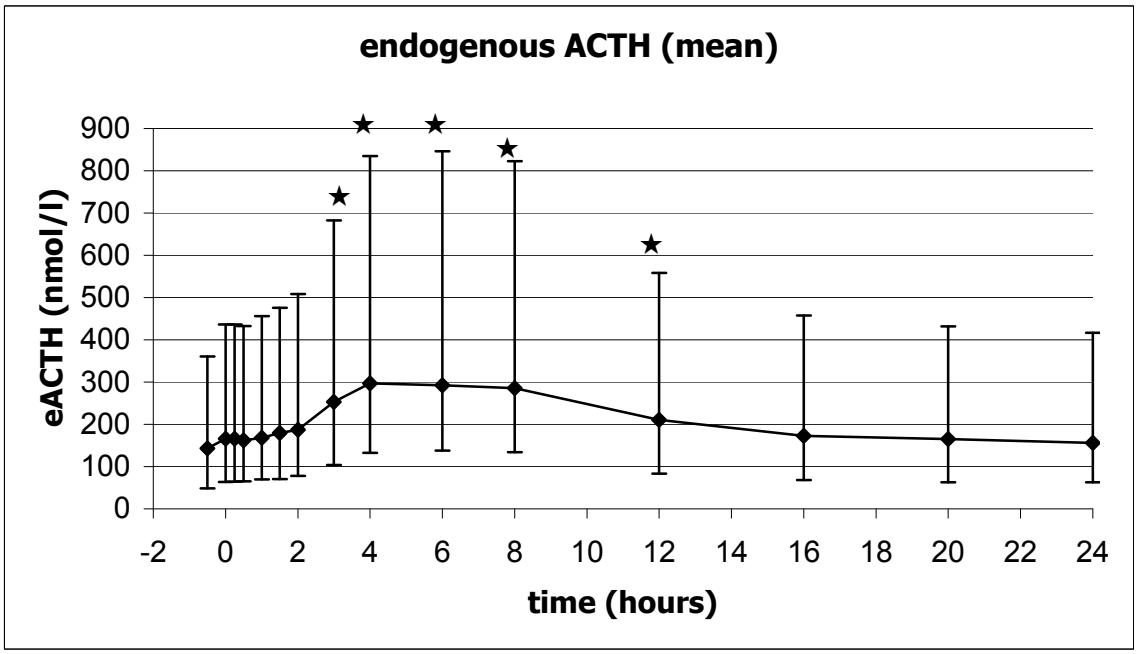
For explanation of graphical display see figure 5.



5. endogenous ACTH

Mean endogenous ACTH plasma levels increased significantly after trilostane application ($P < 0.001$). By Dunnetts comparison, a significant peak was reached between 3 to 12 hours (figure 11) post dosing. After 12 hours mean levels decreased back to baseline levels.

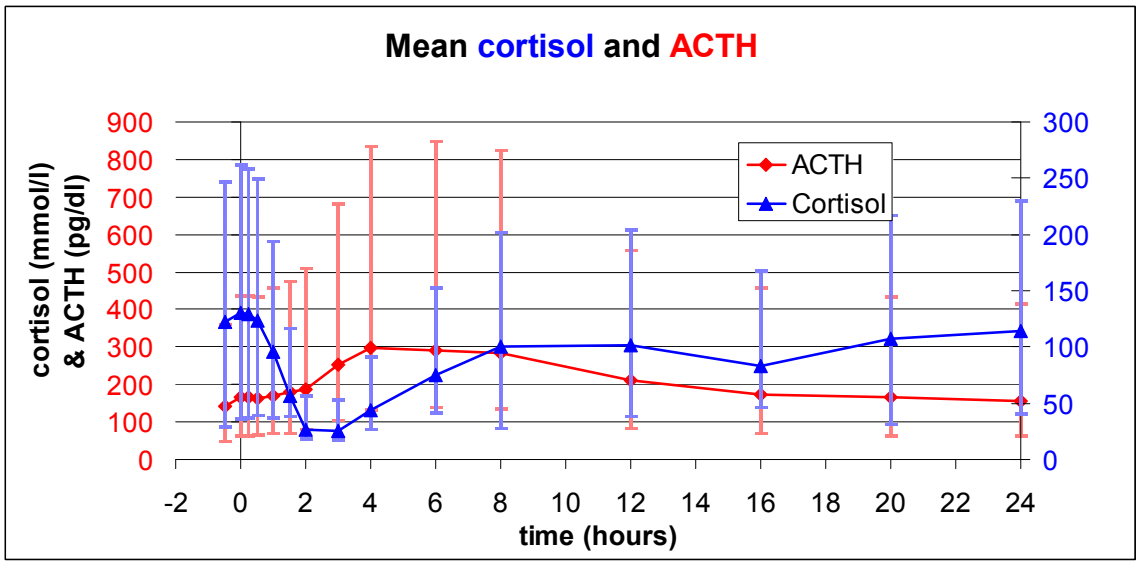
Figure 11: Mean plasma levels of endogenous ACTH in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.
For explanation of graphical display see figure 5.



Comparison of endogenous ACTH and cortisol levels

While serum cortisol levels decreased with minimum levels 3 hours after drug dosing, endogenous ACTH increased after trilostane application and reached a peak level after 4 to 8 hours (figure 12).

Figure 12: Comparison of serum cortisol and plasma endogenous ACTH levels.

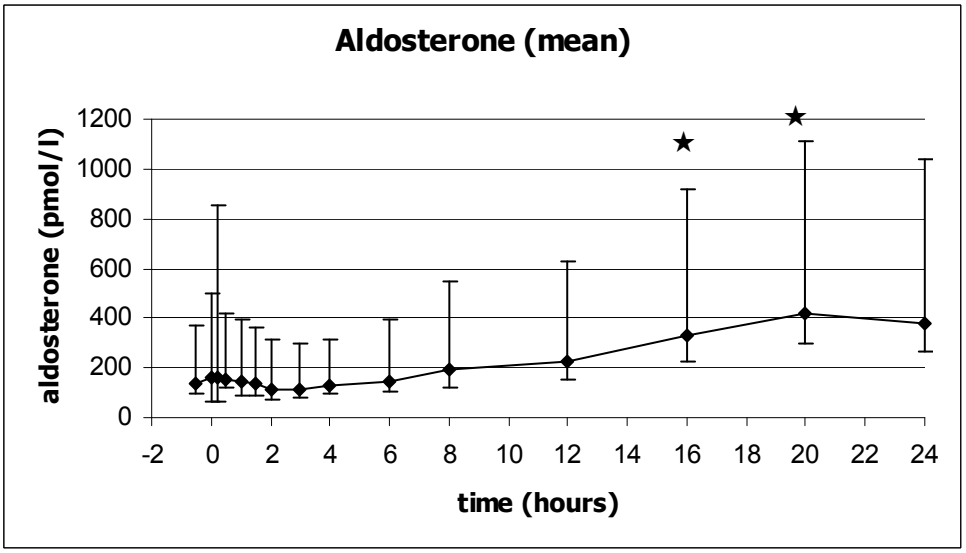


legend:
ACTH = Adrenocorticotropic hormone

6. Aldosterone

In the first hours after trilostane application serum aldosterone levels remained more or less the same in all dogs (figure 13). There was a significant increase in mean serum aldosterone concentration between 16 and 20 hours ($P<0.01$). In two dogs, serum aldosterone levels were below the detection limit of the assay on a few time points throughout the day, however no adverse effects at these time points were seen.

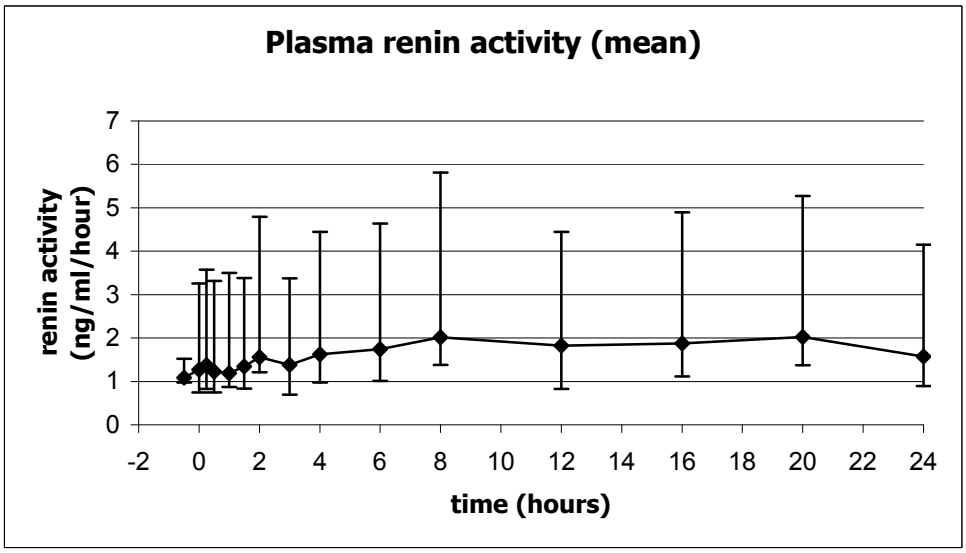
Figure 13: Mean serum aldosterone levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.
For explanation of graphical display see figure 5.



7. Renin

Renin plasma activity levels increased significantly after trilostane application ($P = 0.0002$) and reached peak levels after 8 hours (figure 14). While staying at a stable level for the next 12 hours, renin plasma activity levels decreased slightly 20 hours after trilostane dosing.

Figure 14: Mean plasma renin activity levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.
For explanation of graphical display see figure 5.

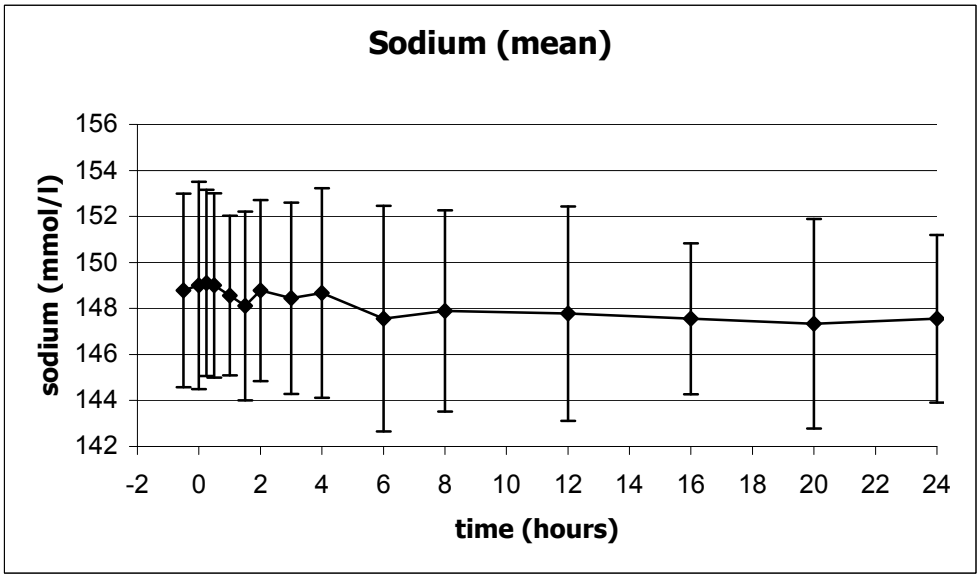


8. Electrolytes

Sodium

Plasma sodium levels remained within the reference range (141–152 mmol/l) throughout the day in all but 4 dogs. Dogs 4, 5, 8 and 10 had short episodes each of mild hypernatraemia shortly before or after trilostane application. No episode of hyponatraemia was seen in any of the dogs. Throughout the study period there was only mild fluctuation of mean serum sodium concentration (figure 15) (P = 0.582), however, there was quite a marked inter-individual variation.

Figure 15: Mean serum sodium levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.
Since data were normally distributed graphical display shows mean results ± standard deviation

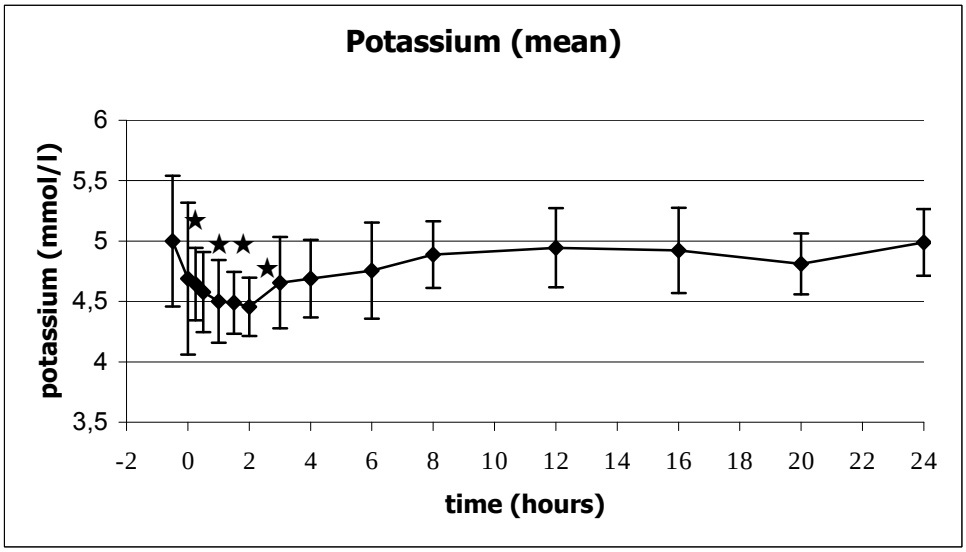


Potassium

There was a significant decrease of mean plasma potassium concentration (figure 16) between 0,5 and 2 hours after trilostane administration ($P < 0.05$). Two dogs had one or two mild episodes of plasma potassium levels above the reference range (3,6 – 5,4 mmol/l) and no dogs became hypokalaemic.

Figure 16: Mean serum potassium levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.

Since data were normally distributed graphical display shows mean results ± standard deviation.



Sodium/Potassium-ratio

Although three dogs showed sodium/potassium-ratios below 27 at varying time points after trilostane application (table 5), none showed any adverse effects.

Table 5: *Sodium/potassium-ratio in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.*
A sodium/potassium ratio of less than 27 was considered a high risk of causing signs of hypoadrenocorticism. Results below this level are written in bold.

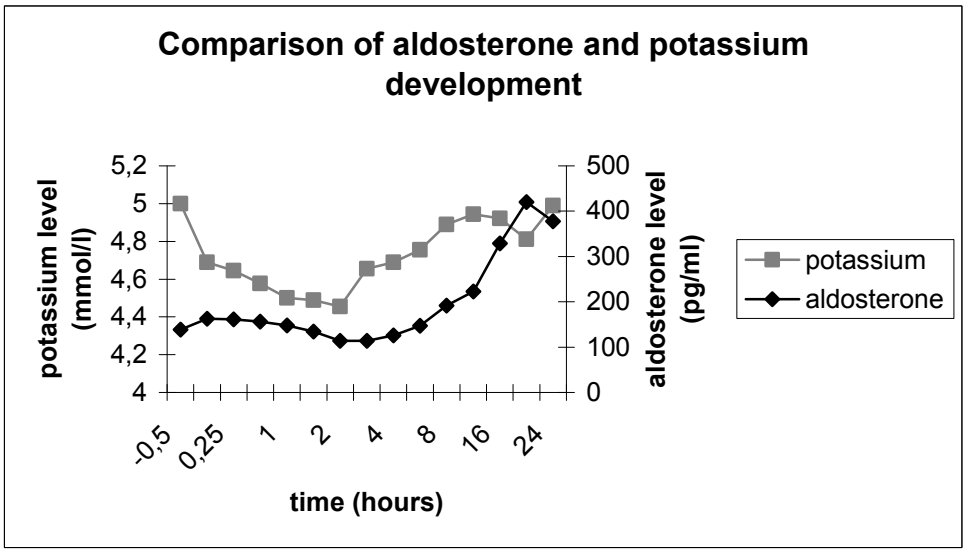
TIME POINTS (hours)	-0.5	0	0.25	0.5	1	1.5	2	3
Dog 1	**25.1**	**23.9**	33.1	34.3	34.3	34.4	32.9	32.9
Dog 2	33.5	30.2	32.4	32.4	31.3	30.9	30.4	31.3
Dog 4	**26.7**	32.5	30.2	31.0	34.3	31.7	32.8	31.7
Dog 5	34.0	32.1	33.0	32.1	30.8	31.5	32.4	33.6
Dog 6	30.0	32.0	30.0	32.6	30.2	32.8	32.1	28.1
Dog 7	29.0	40.0	32.7	31.0	33.3	32.0	34.9	32.9
Dog 8	32.0	32.2	36.3	39.2	38.7	38.5	37.7	35.1
Dog 9	30.2	32.3	32.3	30.4	33.8	33.8	33.8	33.3
Dog 10	29.8	30.0	30.0	31.2	31.7	32.3	34.2	29.4

TIME POINTS (hours)	4	6	8	12	16	20	24
Dog 1	31.4	32.7	30.4	28.5	27.7	29.6	**29.6**
Dog 2	29.5	**26.5**	29.8	28.4	31.5	30.9	30.9
Dog 4	32.0	29.6	29.0	31.4	28.3	30.2	28.3
Dog 5	33.1	32.4	29.4	29.6	31.5	28.8	30.8
Dog 6	32.1	33.6	29.6	29.6	29.8	31.7	30.6
Dog 7	30.4	30.2	29.2	31.7	28.1	29.1	28.9
Dog 8	36.7	36.4	35.1	34.8	32.6	32.8	31.9
Dog 9	31.8	30.9	27.7	28.4	31.1	31.1	27.4
Dog 10	29.3	28.7	33.0	27.6	31.7	31.7	31.2

Potassium/Aldosterone-ratio

While mean plasma potassium levels decreased during the first 2 hours after trilostane application, serum aldosterone levels increased slightly. After this time point, plasma potassium levels showed a slight increase back to initial levels. Serum aldosterone levels increased non-significantly 16 to 20 hours after trilostane dosing (figure 17).

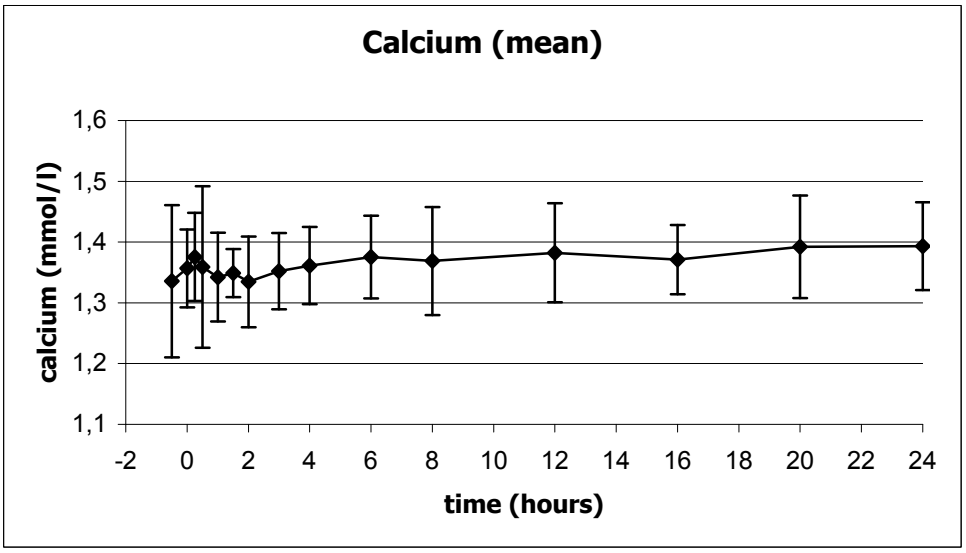
Figure 17: *Comparison of aldosterone and potassium development in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.*



Calcium

Mean plasma calcium levels showed a slight, however non-significant decrease (P = 0.593) shortly after trilostane application with minimum ionized calcium levels 2 hours after drug dosing. Throughout the study period there was only mild fluctuation of mean plasma calcium concentration (figure 18). Plasma calcium levels remained within the reference range (1,2–1,45 mmol/l) in all but one dog throughout the day. Dog 10 was mildly hypercalcaemic ¼ to ½ hour and again 4 hours to 24 hours after trilostane administration. The maximum calcium level reached 1,55mmol/l (12 hours after trilostane application). None of the dogs was hypocalcaemic.

Figure 18: *Mean serum calcium levels in 8 dogs with pituitary-dependent hyperadrenocorticism on day 30 of treatment with trilostane.*
Since data were normally distributed graphical display shows mean results ± standard deviation



VI. Discussion

The signalement of the dogs in this study is comparable to previous reports of canine HAC with the exception of breed (45,51,61). There was an equal sex distribution and similar age range, however while typical breeds are mostly small dogs such as poodles, terriers and dachshounds, (36,51,61) the inclusion criteria of this study dictated a weight of at least 15 kg. Of the breeds reported here, only golden retrievers and beagles are known to develop HAC regularly (47).

Historic complaints and physical findings in these dogs were consistent with the literature (36,45,47) with nine of 10 dogs showing polyuria, polydipsia and/or nocturia. Although no polyuria/polydipsia was noticed in dog 10, it had a low specific gravity of the urine (specific gravity of 1008).

Although well controlled clinically as well as based on the ACTH stimulation tests, dog 3 collapsed unexpectedly and seemed to be unconscious for a few minutes just before the blood sampling procedure on day 30. Shortly after this syncope everything normalised and no further abnormalities could be detected on a full investigation (including radiographs and echocardiography). Because of this incident, the dog was removed from further blood sampling. While, the exact reason of this incident remains unclear a possible explanation might be a thrombus formation, which dissolved spontaneously. Dogs with HAC are known to have a state of hypercoagulability which might result in thromboembolism (31). The reasons are higher levels of procoagulant factors (factors II, V, VII, IX, X, XII) and lower levels of anti-coagulative antithrombin III (31). Hypercoagulability may still happen weeks after starting therapy for HAC. About two years after this episode, the dog continues to receive trilostane, remains healthy and no syncope has happened again.

Clinical improvement was good in all but one dog (dog 9) confirming the efficacy of trilostane for canine PDH (7,41,45,61). Dog 9 had serum cortisol concentrations before and after ACTH stimulation well within the normal range (13,58) but continued to suffer from polyuria and polydipsia. Serum osmolality was decreased, indicating potential hyperhydration as seen in dogs with psychogenic polydipsia (19, 52). After water restriction, the urine specific gravity increased, demonstrating that the dog was able to conserve its urine concentrating potential within the kidneys. Polyuria in dogs with HAC can be due to abnormal ADH-receptor interaction when abnormally high serum cortisol levels are present. However, psychogenic polydipsia is also possible as part of the clinical picture. Additionally, orthopaedic problems worsened under trilostane therapy in dog 9. Because cortisol has an anti-inflammatory effect (35), dogs with arthrosis and painful orthopaedic problems show less clinical symptoms as long as HAC is untreated. Under treatment cortisol blood levels decrease and so does the anti-inflammatory effect. As a result, these patients' orthopaedic symptoms worsen (Neiger; personal observation). After treatment with non-steroidal anti-inflammatory drugs, dog 9 showed improvement in its agility.

Canine HAC results in several typical haematological and biochemical abnormalities, such as a stress leucogram or elevated levels of alkaline phosphatase and cholesterol (47). Irrespective of treatment modality of HAC, several of these abnormalities normalise (33). Similarly, laboratory findings had generally improved on the 30th day

of therapy in the dogs of this study, although some of the parameters still were not within the reference range.

In rats and monkeys trilostane absorption is quick after oral dosing with peak blood concentrations occurring between 0.5-1 hour (rat) and between 2-4 hours (monkey) (57). In human volunteers the peak concentrations are reached after 1 hour (60). Trilostane plasma levels in the dogs of this study with PDH confirm that trilostane uptake is fast and maximum levels are reached after 1.5 hours. Similar results have also been found in clinically healthy beagle dogs given 10 mg/kg trilostane orally and peak plasma trilostane levels measured at 1.5-2 hours later (Arnolds Ltd. - data on file). The decline of trilostane follows a triexponential decrease with trilostane cleared from the blood after 7 hours (rat), 6-8 hours (human) and 48 hours (monkey) (39,57,60). Trilostane clearance from the blood in the dogs of this study is comparably fast and after about 12 hours, no more trilostane is detectable.

In rats ketotrilostane is rapidly formed from trilostane (39). As shown in figure 5, shortly after oral application of trilostane the plasma level of trilostane is much higher than that of ketotrilostane. However, ketotrilostane is formed rapidly and after 0.5 to 1 hour, the ratio starts to shift towards ketotrilostane with one molecule of ketotrilostane formed from one molecule of trilostane. In healthy rats, McGee et al. (39) found that ketotrilostane is formed from trilostane until an equilibrium is reached. Not enough data is available to say wether an equilibrium will be obtained in dogs with PDH but it is clearly visible that maximum trilostane and ketotrilostane plasma levels are reached more or less simultaneously. The reason of a proportional shifts towards ketotrilostane later in the day may be explained due to the facts that ketotrilostane is one of the metabolites of trilostane, as has been shown previously (39), or it might indicate that trilostane is secreted more rapidly than ketotrilostane.

Because ketotrilostane is formed rapidly from trilostane, the peak levels of both compounds are seen at the same time point, 1.5 to 2 hours after drug administration. Although both substances contribute to the drug's effect, ketotrilostane shows a 1.7 times higher activity than trilostane in the inhibition of steroidogenesis (38). This might explain why the time point of the lowest cortisol concentrations is slightly delayed.

The trilostane and ketotrilostane profiles of dog 7 remain unclear. Interestingly, at time point -30 minutes, i.e. half an hour before trilostane was given, both trilostane and ketotrilostane plasma levels below the detection limit of the assays were found. This corresponds with plasma trilostane and ketotrilostane levels at this time point in all other dogs. As such it seems that the erratic profiles of dog 7 might be a single event.

Maximum effect of trilostane on glucocorticoid production is reached 2-4 hours after application of the drug. Accordingly, at this time point minimal cortisol levels can be found (44). As a result, endogenous ACTH levels increase 4 to 8 hours after drug dosing because the negative feedback mechanism which also influences the tumour's secretion of ACTH is removed. In comparison to healthy dogs' reactions, the tumour's answer to the lost negative feedback is exaggerated and prolonged.

Most interestingly, effects of trilostane on cortisol levels can be found long after plasma trilostane levels reach non-detectable levels. This is consistent with previous observations (45). No clear explanation can be given from this study but several mechanisms could be speculated. Firstly, it might be possible that the assays measure only circulating trilostane or ketotrilostane levels while other metabolites, which have an effect of stroidogenesis and are excreted more slowly, are not measured. Secondly, intracellular effects of trilostane or its metabolites on steroid receptor interaction (not only enzyme inhibition) are possible. Or thirdly, prolonged enzyme inhibition by trilostane or its metabolites must also be considered.

Cortisol levels were very low in all dogs and even below the detection limit of the assay in most of the patients on at least one occasion (all in all, on 18 occasions), but none of the dogs showed any adverse effects and all appeared clinically healthy.

Because of the ACTH-producing tumour, plasma ACTH-levels is higher in dogs with PDH than in healthy dogs (47,74). Under therapy, the negative feedback of cortisol is removed and the endogenous ACTH levels of these dogs increase even more. Thus, dogs with PDH undergoing therapy show much higher ACTH levels than healthy dogs or dogs with PDH where treatment has not been initiated, yet (57,66,73). The highest ACTH levels are found at the time point of maximum trilostane effect, 3 hours after drug dosing. All blood samples showed endogenous ACTH levels above the normal range. At maximum endogenous ACTH levels, concentrations of up to 15 times the reference range (20 – 80 pg/ml) could be found.

Because there was quite marked inter-individual variation of endogenous ACTH levels over the 24 hours and in some dogs changes in endogenous ACTH levels were only minimal, measurement of endogenous ACTH is most likely not useful for the evaluation of therapeutical success of trilostane, as has been suggested (6).

Three hours after trilostane intake, at the time point of maximum trilostane effect, aldosterone levels were decreased. In two of the patients, aldosterone concentrations lower than the detection limit of the assay were found. Because trilostane is an inhibitor of the 3ß-hydroxysteroid dehydrogenase, an enzyme which has an effect on the production of all steroid hormones in the adrenal cortex (57), this effect is not surprising. Although aldosterone levels are low, none of the dogs showed any adverse effects.

After its lowest serum concentration, mean aldosterone levels increased again. Aldosterone levels well above the reference range were found. This is consistent with the results of Sieber-Ruckstuhl et al. (66) and in contrast to a study by Wenger et al. (71) who found aldosterone levels unaltered. On the other hand, both noticed that trilostane's effect on serum aldosterone concentration is less pronounced than on serum cortisol concentration. Although only playing a minor role, ACTH is nevertheless known to influence serum aldosterone levels (25). Therefore, long-term excessive amounts of endogenous ACTH – as seen in dogs with PDH - may lead to increased serum aldosterone levels as long as the effect of trilostane does not lower them.

Eight to 20 hours after trilostane administration, renin plasma activity is significantly increased (8h: $p < 0.01$; 12h to 20h: $p < 0.05$). Since peak aldosterone concentrations are found 16 to 24 hours (16h: $p < 0.05$; 20h to 24h: $p < 0.01$) after drug dosing, renin may also have an effect on this steroid hormone.

Renin plasma activity shows a fidgety profile in the first hours after trilostane uptake which is consistent with the maximum effect of trilostane. At this time point, aldosterone reaches its lowest concentration. Renin may increase because of a decrease of blood flow to the kidneys second to low aldosterone levels and, therefore, increased loss of fluids via the urine. This represents one of the two main mechanisms through which renin activity is regulated. The second trigger of an increase of renin activity is a high level of plasma potassium. Surprisingly, at the time point of maximum effect of trilostane, potassium levels were decreased. Thus, this should cause low renin activity levels.

Potassium levels show a surprising development. At times when aldosterone was at its lowest concentration, potassium levels were low, but nevertheless well inside the reference range. Wenger et al. (25) have noticed recently that there seems to be no correlation between serum concentrations of aldosterone and potassium during treatment. Because aldosterone causes an active secretion of K̆-ions, at time points of low aldosterone levels, high potassium concentrations would have been expected. Thus, there has to be at least one other mechanism that regulates the plasma potassium concentration. A direct effect of trilostane is possible. Because trilostane is a competitive inhibitor of enzymes situated in glucocorticoid and mineralcorticoid biosynthesis, it may also have a partially agonistic effect. The chemical structure of trilostane is in parts similiar to the one of steroid hormones. Thus, trilostane may have a mineralcorticoid effect and it may directly cause the decrease of potassium levels 3-4 hours after trilostane uptake. This also is the time point when maximum trilostane concentrations can be found. However, Pott et al. (57) found no direct or indirect hormonal activity of trilostane in rats.

Another hypothesis was developed recently by Sieber-Ruckstuhl et al. (66); they noticed that, although low aldosterone levels were measured both after mitotane as well as trilostane treatment, dogs which were given mitotane regularly showed signs of aldosterone deficiency while dogs on trilostane treatment still appeared healthy. The authors conclude that steroid precursors accumulate because of the blockade of 3ß-hydroxysteroid dehydrogenase and supposedly other enzymes may also have mineralcorticoid effects. This seems plausible since both mitotane and trilostane use completely different mechanisms to decreased cortisol levels. Thus, if these precursors not only have cortisol, but also some mineralcorticoid effect, this may explain why potassium levels are low when, at the same time, aldosterone levels are very low, too.

This potassium-lowering effect would have to be very strong: it should not only decrease potassium levels, but also compensate the effect of low aldosterone levels which by itself causes increased levels of potassium. As such, it might be that both mechanisms work complementarily and lead to the decrease of serum potassium.

If decreased aldosterone would have a significant effect, one might be able to find abnormally low sodium:potassium-ratios. This ration was above 27 on all but three occasions throughout the day. Since serum potassium levels were rather decreased than increased and serum sodium concentration remained stable throughout the day, it seems that problems pointing towards a hypoadrenocorticoid abnormality are uncommon in dogs with PDH on trilostane. That hypoadrenocorticoid crisis can still be found clinically (8,14,45) might be more likely due to an adrenal necrosis which can be the result from long-standing increased levels of ACTH (8) than to abnormally low aldosterone levels in these dogs.

In summary, data obtained in this study confirms that trilostane is an effective drug in the treatment of canine PDH. Marked aldosterone abnormalities throughout the day could not be seen and serum potassium level rather decreases than increases shortly after trilostane administration. Some of the changes in hormones and electrolyte levels remain unclear, especially the long-term decrease of cortisol despite the short plasma levels of trilostane or ketotrilostane. Therefore, further studies are needed to understand the effect of trilostane better.

VII. Summary

Ten dogs with PDH took part in this study. After confirming the diagnosis, treatment with once daily trilostane was started. On their 10th and 30th day of therapy, the dogs were controlled clinically and an ACTH stimulation test was performed 3 to 4 hours after trilostane dosing. On the 30th day of therapy, the dogs were hospitalized and over 24 hours, blood samples were taken for the measurement of trilostane, ketotrilostane, endogenous ACTH, cortisol, aldosterone, renin and serum electrolytes. On the 10th and 30th day of therapy, clinical improvement was good in all but one dog. Laboratory findings including the ACTH stimulation test results had generally improved on the 30th day of therapy, although some of the parameters still were not within the reference range. None of the patients showed any adverse effects. Nevertheless, on the 30th day of therapy, one of the dogs collapsed suddenly and, although regenerating quickly, was removed from further blood sampling.

Trilostane absorption was quick and ketotrilostane was formed immediately. For both substances, peak concentrations were reached 1.5 to 3 (4) hours after trilostane administration. Accordingly, lowest serum cortisol and aldosterone levels were found at nearly the same time points. Although cortisol and aldosterone levels were very low in all dogs, none showed any adverse effects.

Because the negative feedback mechanism caused by high cortisol levels was removed, all dogs showed highest endogenous ACTH levels 4 to 8 hours after trilostane dosing. At all time points, all dogs had endogenous ACTH levels well above the reference range. Because there was quite marked inter-individual variation over the 24 hours and in some dogs changes in endogenous ACTH levels were only minimal, its measurement is most likely not useful for the evaluation of therapeutical success of trilostane.

Serum renin activity remained rather unchanged during the day. While changes in serum sodium and calcium were insignificant, serum potassium showed some astonishing development: At times when aldosterone was at its lowest concentration and high potassium levels would be expected, potassium levels were low, but nevertheless well inside the reference range. An aldosterone-like effect of trilostane or of accumulating steroid precursors seems possible, although further studies are necessary to verify these hypotheses. This may be one of the reasons why trilostane is more secure in the treatment of PDH than lysodren.

VIII. Zusammenfassung

Zehn Hunde mit hypophysärem Hyperadrenokortizismus nahmen an dieser Studie teil. Nachdem die Diagnose gesichert wurde, begann die Therapie mit einmal täglich verabreichtem Trilostan. Die Hunde wurden am 10ten und 30ten Tag der Therapie sowohl klinisch als auch mittels ACTH-Stimulationstest (3 bis 4 Stunden nach der Gabe von Trilostan) kontrolliert. Am 30ten Tag der Therapie wurden die Tiere stationär aufgenommen und über 24 Stunden Blutproben genommen zur Bestimmung von Trilostan, Ketotrilostan, endogenem ACTH, Kortisol, Aldosteron, Renin und Serumelektrolyten.

Am 10ten und 30ten Tag der Behandlung zeigten alle Hunde außer einem ein gutes klinisches Ansprechen auf die Therapie. Die Ergebnisse der Laboruntersuchungen inklusive der durchgeführten ACTH-Stimulationstests ergaben grundsätzlich bessere Werte, auch wenn nicht alle Resultate den Normbereich erreichten. Keiner der Patienten zeigte Nebenwirkungen. Dennoch kollabierte einer der Hunde plötzlich am 30ten Tag der Therapie, und obwohl er sich sehr schnell wieder regenerierte, wurde er aus der Studie entfernt.

Die Absorption von Trilostan geschah schnell und Ketotrilostan wurde direkt gebildet. Höchste Konzentrationen beider Substanzen waren 1,5 bis 3 (4) Stunden nach der Gabe von Trilostan nachweisbar. Entsprechend wurden die niedrigsten Werte des Serumkortisols und des Serumaldosterons zum annähernd gleichen Zeitpunkt gefunden. Obwohl die Konzentrationen des Kortisol und des Aldosterons in allen Hunden sehr niedrig waren, zeigte keiner der Patienten Nebenwirkungen.

Nach Wegfall des negativen Rückkopplungsmechanismus' durch hohe Kortisolwerte zeigten alle Hunde höchste Werte des endogenen ACTH 4 bis 8 Stunden nach Tablettengabe. Zu allen Zeitpunkten hatten alle Hunde endogene ACTH-Werte weit über dem Normalbereich. Da sich eine starke Variation der Werte zwischen den Individuen während der 24 Stunden nachweisen ließ und einige Hunde nur minimale Veränderungen der Konzentration des endogenen ACTH zeigten, erscheint die Messung des endogenen ACTH nicht nützlich zur Überprüfung des Therapieerfolges von Trilostan.

Die Serumreninaktivität blieb mehr oder weniger unverändert. Während sich auch die Konzentrationen der Serumelektrolyte Natrium und Kalzium nicht signifikant veränderten, wies Kalium eine erstaunliche Entwicklung auf: Zum Zeitpunkt der niedrigsten Aldosteronwerte, wenn entsprechend eher erhöhte Kaliumwerte zu erwarten wären, fanden sich niedrige Kaliumwerte, wenn auch noch innerhalb des Normalbereiches. Ein Aldosteron-artiger Effekt des Trilostans oder aber der sich bildenden Steroidvorläufersubstanzen wäre denkbar, auch wenn diese Hypothesen erst durch weitere Studien bewiesen werden müssen. Dies mag (unter anderem) die im Vergleich zum Lysodren bessere Verträglichkeit von Trilostan erklären.

IX. Acknowledgements

I want to thank everyone who has supported me along the way.
Special thanks go to Arnolds Ltd., and especially to Emma Chapman, who made this study possible. I also owe thanks to CSL and HFL laboratories for measuring the blood parameters.
Prof. Dr. Reto Neiger has not only helped me with the practical aspects of this study, but he also provided useful information about HAC in general, let me take part in his own huge experiences with trilostane and was always there to talk to dogs' owners or to interpret unclear laboratory test results.
I also want to thank my colleagues at the Small Animal Clinic (Internal Medicine), Gießen, for supplying me with cases of PDH, helping me collecting blood samples and generally cheering me up when no cases of PDH happened to come along for a very long time.
I also got help from the Small Animal Clinic (Surgery), Gießen; my colleagues there performed various ultrasound examinations for me and tried to locate every adrenal gland I asked them to.
The people working at the laboratory of the Small Animal Clinic, Gießen, helped me to get the blood work of my patients done. Elisabeth Jüngst-Carter was there for all my questions und always gave me an encouraging smile when things did not work out the way I planed.
10 dogs and their owners participated in this study; although most of them had a long way to drive each time, they came in for every examination that was necessary and left their pet at the clinic for more than 24 hours to give me the possibility to collect samples. I have to thank them for their trust.
Last but not least, I want to thank my family for their support not only during this study but also before and after. My mother, my father, my grandmother and my sister Sonja always believed in me and supported me the best they could. I'm very grateful to have such wonderful people around me! I want to thank Dr. Christoph Clemen who I very much enjoyed discussing my results with. I also have to thank Tim Spangenberg for his support.
Without all the people mentioned above, this study would not have been possible!

X. Laboratories

α = Alomed laboratory, Öschlestr. 77, 78315 Radolphzell, Germany

β = CSL, Kingfisher Way, Silverlink Business Park, Wallsend, Tyne & Wear NE28 9NX, UK

γ = HFL Ltd., Newmarket Road, Fordham, Cambridgeshire CB75WW, UK

XI. References

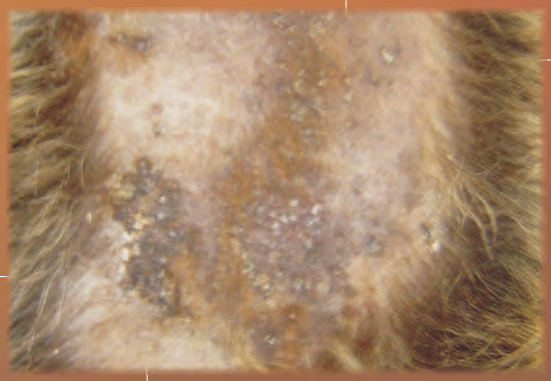
1. Baker JF, Benzinger D, Chalecki BW, Clemans S, Fritz A, O'Melia PE, Shargel L, Edelson J. Disposition of trilostane in the rat and monkey. Arch Int Pharmacodyn Ther 1980; 243: 4-16.
2. Barker EN, Campbell S, Tebb AJ, Neiger R, Herrtage ME, Reid SWJ, Ramsey JK. A comparison of the survival times of dogs treated with either mitotane or trilostane for pituitary dependent hyperadrenocorticism. Proceedings of the annual meeting of the British Small Animal Veterinary Association, Birmingham, UK; 2004.
3. Barthez PY, Nyland TG, Feldman EC. Ultrasonographic evaluation of the adrenal glands in dogs. J Am Vet Med Assoc 1995; 207: 1180-3
4. Beardwell CG, Hindley AC, Wilkinson PM, St John J, Bu'lock D. Hormonal changes in postmenopausal women with breast cancer treated with trilostane and dexamethasone. Clin Endocrinol (Oxf) 1985; 23: 413-21
5. Braddock JA, Church DB, Robertson ID, Watson AD. Trilostane treatment in dogs with pituitary-dependent hyperadrenocorticism. Aust Vet J 2003; 81: 600-7.
6. Buijtels JJCWM, Galac S, Kooistra HS. Measurement of plasma ACTH concentration to determine the optimal dose of trilostane in dogs with pituitary-dependent hyperadrenocorticism. 14th ECVIM congress, Uppsala, Sweden 2003
7. Campbell S, Trettien A, Kozan B. A noncomparative open-label study evaluating the efficacy of selegeline hydrochloride in a clinical setting. Vet Therap 2001; 2: 24-39
8. Chapman PS, Kelly DF, Archer J, Brockman DJ, Neiger R. Adrenal necrosis in a dog receiving trilostane for the treatment of hyperadrenocorticism. J Small Anim Pract 2004; 45: 307-10
9. Chastain CB, Franklin RT, Ganjam VK, Madsen RW. Evaluation of the hypothalamic pituitary-adrenal axis in clinically stressed dogs. J Am Anim Hosp Assoc 1986; 22: 435-41
10. Chu PS, Buzdar AU, Hortobagyi GN. Trilostane and hydrocortisone in treatment of metastatic breast cancer. Breast Cancer Res Treat 1989; 13: 117-21
11. Coombes RC, Powles TJ, Muindi J, Hunt J, Ward M, Perez D, Neville AM. Trilostane therapy for advanced breast cancer. Cancer Treat Rep 1985; 69: 351-4
12. Dewis P, Anderson DC, Bu'lock DE, Earnshaw R, Kelly WF. Experiences with trilostane in the treatment of Cushing's Syndrome. Clin Endocrinol (Oxf) 1983; 18: 533-40

13. Dunn KJ, Herrtage ME, Dunn JK. Use of ACTH stimulation tests to monitor the treatment of canine hyperadrenocorticism. Vet Rec 1995; 137: 161-5
14. Eastwood JM, Elwood CM. Prolonged hypoadrenocorticism in five dogs treated with trilostane for pituitary-dependent hyperadrenocorticism (PDH). Proceedings of the annual meeting of the British Small Animal Veterinary Association, Birmingham, UK, 2003
15. Eastwood JM, Elwood CM, Hurley KJ. Trilostane treatment of a dog with a functional adrenocortical neoplasia. J Small Anim Pract 2003; 44: 126-31
16. Feldman EC, Bruyette DS. Plasma cortisol response to ketoconazole administration in dogs with hyperadrenocorticism. J Am Vet Med Assoc 1990; 197: 71-8
17. Feldman EC, Nelson RW, Endocrinology and reproduction, textbook, WB Saunders, Philadelphia, USA, 2004
18. Fox B. Venous infarction of the adrenal glands. J Pathol 1976; 119: 65-89 **45**
19. Gebel F, Meng H, Michot F, Truniger B. Psychogenic water intoxication. Schweiz Med Wochenschr 1989; 119:169-77
20. Golden DL, Lothrop CD. A retrospective study of aldosterone secretion in normal and adrenopathic dogs. J Vet Intern Med 1986; 2: 121-XX
21. Goosens MMC, Feldman EC, Theon AP, Koblik PD. Efficacy of cobalt 60 radiotherapy in dogs with pituitary-dependent hyperadrenocorticism. J Am Vet Med Assoc 1998; 212: 374-6
22. Gould SM, Baines EA, Marnion PA, Evans H, Herrtage ME. Use of endogenous ACTH concentration and adrenal ultrasonography to distinguish the cause of canine hyperadrenocorticism. J Small Anim Pract 2001; 42: 113-21
23. Griffing GT, Melby JC. Reversal of diuretic-induced secondary hyperaldosteronism and hyperkalemia with trilostane, an inhibitor of adrenal steroidogenesis. Metabolism 1989; 38: 353-6
24. Grooters AM, Biller DS, Theisen SK, Miyabayashi T. Ultrasonographic characteristics of the adrenal glands in dogs with pituitary-dependent hyperadrenocorticism: Comparison with normal dogs. J Vet Int Med 1996; 10: 110-5
25. Guyton AC. Textbook of medical physiology, textbook, W.B. Saunders Company, Philadelphia, USA 1991
26. Hardie EM, Vaden SL, Spaulding K, Malarkey DE. Splenic infarction in 16 dogs: A retrospective study. J Vet Int Med 1995; 9: 141-8
27. Hick C, Hick A. Physiologie, textbook, Gustav Fischer Verlag, Stuttgart, Germany 1998
28. Hurley KJ. Trilostane in the treatment of canine hyperadrenocorticism. Proceedings of the ESVIM congress, Vienna, Austria; 1998
29. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. J Am Vet Med Assoc 1998; 212: 369-73
30. Ingle JN, Krook JE, Schaid DJ, Everson LK, Mailliard JA, Long HJ, McCormack GW. Evaluation of trilostane and hydrocortisone in women with metastatic breast cancer and prior hormonal therapy exposure. Am J Clin Oncol 1990; 13(2): 93-7
31. Jacoby RC, Owings JT, Ortega T, Gosselin R, Feldman EC. Biochemical basis for the hypercoagable state seen in Cushing syndrome. Arch Surg 2001; 136: 1003-6

32. Javadi S, Koistra HS, Mol JA, Boer P, Boer WH, Rijnbeck A. Plasma aldosterone concentration and plasma renin activity in healthy dogs and in dogs with hyperadrenocorticism. Vet Rec 2003; 153: 521-5
33. Kaplan AJ, Peterson ME, Kemppainen RJ. Effects of disease on the results of diagnostics tests for the use in detecting hyperadrenocorticism in dogs. J Am Vet Med Assoc 1995; 207: 445-50
34. Komanicky P, Spark RF, Melby JC. Treatment of Cushing's Syndrome with trilostane, an inhibitor of adrenal steroid biosynthesis. J Clin Endocrinol Metab 1978; 47: 1042-51
35. Lewis GP, Piper PJ. Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids. Nature 1975; 254: 308-11
36. Ling GV, Staubenfeldt GH, Comer KM, Gribble DH, Schechter RD. Canine hyperadrenocorticism: Pretreatment clinical and laboratory evaluation of 117 cases. J Am Vet Med Assoc 1979; 174:1211-5
37. Mantis B, Lamb CR, Witt AL, Neiger R. Changes in ultrasonographic appearance of adrenal glands in dogs with pituitary-dependent hyperadrenocorticism treated with trilostane. Vet Radiol Ultrasound 2003; 44: 682-5
38. McGee JP, Palin KJ, Shaw PN, Potter C. High-performance liquid chromatographic analysis of trilostane and ketotrilostane in rat plasma. J Chromatogr 1991; 567: 282-7
39. McGee JP, Shaw PN. The pharmacogenetics of trilostane and ketotrilostane in an interconverting system in the rat. Pharm Res 1992; 9: 464-8
40. Meji BP. Hypophysectomy as a treatment for canine and feline Cushing's disease. Vet Clin North Am Small Anim Pract 2001; 31: 1015-41
41. Melville-Walker S. The use of trilostane in the treatment of canine hyperadrenocorticism: Laboratory findings, owner's perspectives and side effects. Elective student project, Royal Veterinary College, London 2002
42. Nakada T, Kazama T, Koike A, Yoshikawa M, Ishikawa S, Katayama T. Primary aldosteronism treated by trilostane. Urology 1985; 25: 207-14
43. Neiger R. New treatment for Cushing's Disease. Proccedings of the ACVIM forum, Seattle, USA; 2002
44. Neiger R, Campbell E. 24-hour cortisol values after trilostane therapy in dogs with hyperadrenocorticism. Proceedings of the 10th ESVIM Congress 2000; 31
45. Neiger R, Ramsey I, O'Connor J, Hurley KJ, Mooney CT. Trilostane treatment of 78 dogs with pituitary-dependent hyperadrenocorticism. Vet Rec 2002; 150: 799-804
46. Neiger R, Witt AL, Noble A, German AJ. Trilostane therapy for treatment of pituitary-dependent hyperadrenocorticism in 5 cats. J Vet Int Med 2004; 18: 160-4
47. Nelson RW, Couto CG. Small animal internal medicine, textbook, Mosby, St. Louis, USA 2003
48. Nichols R. Concurrent illness and complications associated with canine hyperadrenocorticism. Seminars in Veterinary Medicine and Surgary (Small Animal) 1994; 9: 132-6
49. Nomura K, Demura H, Imaki T, Miyagawa M, Ono M, Yano T, Shizume K. Concomitant falls of plasma cortisol and ACTH levels in a case of Cushing's

disease during treatment with trilostane. Acta Endocrinol (Copenh) 1984; 105: 93-8
50. Nomura K, Demura H, Horiba N, Shizume K. Long-term treatment of idiopathic hyperaldosteronism using trilostane. Acta endocrinol (Copenh) 1986; 113: 104-10
51. Nothelfer HB, Weinhold K. Formale Pathogenese, Durchschnittsalter und Rassenverteilung im Vergleich 61 Lysodren-behandelter und 36 unbehandelter Fälle von caninem Hyperadrenokortizismus, die in den Jahren 1975 bis 1991 am Institut für Veterinär-Pathologie der Freien Universität Berlin seziert wurden. Berl Münch Tierärztl Wschr 1992; 105: 305-11
52. Oz B, Olmez N, Memis A, Oruk G. Differential diagnosis of polyuria and polydipsia in a patient with spinal cord injury. Am J Med Rehabil 2005; 84; 817-20
53. Park BW, Kim KS, Heo MK, Yang WI, Kim SI, Kim JH, Kim GE, Lee KS. The changes of estrogen receptor-beta variants expression in breast carcinogenesis. J Surg Oncol 2006; 93: 504-10
54. Pedersen HD, Olsen LH, Arnardottir H. Breed differences in the plasma renin activity and plasma aldosterone concentrations of dogs. Zentralbl Veterinarmed A 1995; 42: 435-41
55. Peterson ME, Kintzer PP. Medical treatment of pituitary-dependent hyperadrenocorticism. Mitotane. Vet Clin North Am Small Anim Pract 1997; 27: 255-72
56. Perez AM, Guerrero B. Use of aminoglutethimide in the treatment of pituitary-dependent hyperadrenocorticism in the dog. J Small Anim Pract 2002; 43: 104-8
57. Potts GO, Creange JE, Hardomg HR, Schane HP. Trilostane, an orally active inhibitor of steroid biosynthesis. Steroids 1978; 32: 257-67
58. Reusch CE, Hahnle B. Laboratory parameters for the control of the course of therapy of canine Cushing's syndrome. Tierarztl Prax 1991; 19: 102-6
59. Reusch CE, Steffen T, Hörauf A. The efficacy of L-deprenyl in dogs with pituitary-dependent hyperadrenocorticism. J Vet Int Med 1999; 13: 291-301
60. Robinson DT, Earnshaw RJ, Mitchell R, Powles P, Andrews RS, Robertson WR. The bioavailability and metabolism of trilostane in normal subjects; a comparative study using high pressure liquid chromatographic and quantitative cytochemical assays. J Steroid Biochem 1984; 21: 601-5
61. Ruckstuhl NS, Nett CS, Reusch CE. Results of clinical examination, labaratory tests, and ultrasonography in dogs with pituitary-dependent hyperadrenocorticism treated with trilostane. Am J Vet Res 2002; 63: 506-12
62. Saito J, Nishikawa T. Clinical usefulness of pharmacological treatment of Cushing's syndrome. Nippon Rinsho 1994; 52(3): 793-6
63. Schwedes C, Müller W. Bestimmung des endogenen ACTH beim Hund. Tierärztl Prax 2000; 28 (K): 65-70
64. Semple CG, Beastall GH, Gray CE, Thomson JA. Trilostane in the management of Cushing's syndrome. Acta Endocrinol (Copenh) 1983; 102: 107-10
65. Semple CG, Weir SW, Thomson JA, Beardstall GH. Trilostane and the normal hypothalamus-pituitary-testicular axis.
66. Sieber-Ruckstuhl NS, Boretti FS, Wenger M, Maser-Gluth C, Reusch CE. Cortisol, aldosterone, cortisol precursor, androgen and endogenous ACTH

concentrations in dogs with pituitary-dependent hyperadrenocorticism treated with trilostane. Domest Anim Endocrinol 2006; in press
67. Skelly BJ, Petrus D, Nicholls PK. Use of trilostane for the treatment of pituitary-dependent hyperadrenocorticism in a cat. J Small Anim Pract 2003; 44: 269-272
68. Tidholm A, Haggstrom J, Hansson K. Effects of dilated cardiomyopathy on the renin-angiotensin-aldosterone system, atrial natriuretic peptide activity and thyroid hormone concentrations in dogs. Am J Vet Res 2001; 62: 961-7
69. Tueni E, Devleeschouwer N, Leclercq G, Nijs M, Loune A, Vermeulen A, Paridoers R. Endocrine effects of trilostane; in vitro and in vivo. Eur J Cancer Clin Oncol 1987; 23: 1461-7
70. Ward PD, Carter G, Banks R, MacGregor G. Trilostane as cause of Addisonian crisis. Lancet 1981; 2: 1178
71. Wenger M, Sieber-Ruckstuhl NS, Müller C, Reusch CE. Effect of trilostane on serum concentrations of aldosterone, cortisol, and potassium in dogs with pituitary-dependent hyperadrenocorticism. Am J Vet Res 2004; 65: 1245-50
72. Williams CJ, Barley VL, Blackledge GR, Rowland CG, Tyrell CJ, Bachelot F. Multicentre cross-over study of aminoglutethimide and trilostane in advanced postmenopausal breast cancer. Clin Oncol (R Coll Radiol) 1995; 7(2): 87-92
73. Wintersberg B, Vetter W, Groth H, Greminger P, Vetter H. Primary aldosteronism treated with trilostane. Cardiology 1985; 72 Suppl. 1: 117-21
74. Witt AL, Neiger R. Endogenous plasma adrenocorticotropic hormone before and after therapy with trilostane for pituitary-dependent hyperadrenocorticism. Vet Rec 2004; 154: 399-400



édition scientifique
VVB LAUFERSWEILER VERLAG

VVB LAUFERSWEILER VERLAG
STAUFENBERGRING 15
D-35396 GIESSEN

Tel: 0641-5599888 Fax: -5599890
redaktion@doktorverlag.de
www.doktorverlag.de

ISBN 3-8359-5126-2

9 783835 951266