Primary hyperaldosteronism in cats: expanding the diagnostic net

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Primair hyperaldosteronisme bij katten: uitbreiding van de diagnostische mogelijkheden

(met een samenvatting in het Nederlands)

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Voor mijn ouders
Voor Tom, Alwin en Ilse

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Aims and scope of the thesis

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Primary hyperaldosteronism, also termed primary aldosteronism, low-renin hyperaldosteronism, or Conn's syndrome, is an adrenocortical disorder characterized by autonomous hypersecretion of aldosterone. In 1983, nearly 30 years after the first reported case in humans, primary hyperaldosteronism was first reported to have occurred in a cat. It was initially thought to be rare in this species, but the number of reported cases has risen considerably over the past 15 years, and increased awareness of the disease will probably lead to a further increase in recognized cases.

The mineralocorticoid excess in primary hyperaldosteronism, originating from unilateral or bilateral neoplasia or bilateral hyperplasia of the adrenal zona glomerulosa, results in increased sodium and water retention and enhanced renal excretion of potassium, and thereby can result in systemic arterial hypertension or potassium depletion, or both. Cats with primary hyperaldosteronism are thus typically presented with muscle weakness due to hypokalemic myopathy and/or complications of arterial hypertension, such as acute blindness associated with retinal detachment and/or intraocular hemorrhage.

The general introduction (**Chapter 2**) reviews the anatomy of the adrenal gland, the synthesis, actions, metabolism and regulation of aldosterone, and the pathophysiology, symptoms and signs, diagnosis and therapy of primary hyperaldosteronism in cats. Similarities and differences in the diagnostic approach in humans are also delineated.

Apart from exerting a classic mineralocorticoid effect on epithelia in target organs such as the kidneys, colon and salivary glands, aldosterone also has profound effects on other tissues. Prolonged aldosterone excess can lead to fibrosis and proliferation of endothelial and smooth muscle cells in organs such as the heart and kidneys. There is increasing evidence that these non-epithelial actions of aldosterone can promote and accelerate progressive kidney disease in humans. Chronic renal disease is also relatively common in cats and has been associated with both systemic arterial hypertension and hypokalemia. However, the role of the renin-angiotensin-aldosterone system in the pathogenesis of arterial hypertension and hypokalemia in cats with chronic renal disease is unclear. **Chapter 3** describes cats with apparently non-tumorous, low-renin hyperaldosteronism that was considered to mediate renal failure.

Primary hyperaldosteronism is a potentially curable disease and its treatment, either surgically or pharmacologically, might delay or halt progression of concurrent chronic kidney disease. It was therefore of interest to examine cats with chronic kidney disease for inappropriate aldosterone secretion. In **Chapter 4** its prevalence in a group of cats with chronic kidney disease is reported, using the plasma aldosterone-to-renin ratio as a case-finding test.

Even though cats with systemic arterial hypertension, hypokalemia and/or chronic kidney disease are commonly encountered in private veterinary practice, the aldosterone-to-renin ratio is rarely assessed in this setting. Veterinary practitioners might be discouraged from doing so because of the cumbersome sampling and shipping procedures required to preserve renin activity. An alternative means of diagnosing primary hyperaldosteronism might be the measurement of the urinary aldosterone-to-creatinine ratio. This provides an integrated measure of aldosterone secretion over time, as opposed to the single point assessment of the aldosterone-to-renin ratio. It has the additional advantage that the urine sample for measurement of aldosterone does not have to be frozen immediately and can be collected quite easily. The urinary aldosterone-to-creatinine ratio in healthy cats is reported in **Chapter 5**.

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Ideally, the autonomous hypersecretion of aldosterone in suspected primary hyperaldosteronism should be confirmed by a suppression test. Several such tests have been developed in human medicine, utilizing captopril stimulation, fludrocortisone suppression, saline infusion or oral sodium loading, but no test had been validated for confirmation of primary hyperaldosteronism in cats. A test employing a suppressive agent that reduces the urinary aldosterone-to-creatinine ratio in healthy cats but has little or no effect in those with primary hyperaldosteronism would seem to be the best means of confirming the diagnosis. To investigate this possibility, suppression tests were first carried out in healthy cats, employing either sodium chloride or fludrocortisone acetate. The results, presented in **Chapter 5**, demonstrated that the oral administration of fludrocortisone acetate results in a considerable suppression of the urinary aldosterone-to-creatinine ratio in healthy cats.

The next step, as described in **Chapter 6**, was to evaluate the efficacy and safety of the oral fludrocortisone suppression test for confirmation of the diagnosis of primary hyperaldosteronism in cats with hypokalemia or arterial hypertension or both. Changes in urinary aldosterone excretion were monitored from day to day to determine the minimum duration of the test. Side effects such as a transient decrease in the plasma potassium concentration or a rise in arterial blood pressure were also monitored.

Each feline patient with suspected primary hyperaldosteronism seems to present special clinical and diagnostic challenges. Clinical decisions may have to be made that will impair the conditions for the required diagnostic tests. On the other hand, optimizing the testing conditions, such as by withdrawing spironolactone or antihypertensive medication, may put the patient at risk. This duality, the conflicting requirements for optimal testing conditions and optimal care for the patient, is well illustrated in the case report presented in **Chapter 7**.

In **Chapter 8** the results of the studies described in this thesis are summarized and discussed.

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02

General introduction

Part of the general introduction has been published:

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Adrenal anatomy

The adrenal glands are paired structures that are positioned against the roof of the abdomen (Dyce et al., 2010). They are firm, yellowish-white, asymmetrical, oval-discoid structures that lie in the retroperitoneal fat craniomedial to each kidney. They are in intimate association with the aorta (for the left adrenal) and caudal vena cava (for the right adrenal), and the phrenicoabdominal vein leaves a mild indentation in their ventral surface (Frewein, 1994).

The adrenal glands consist of an outer cortex and an inner medulla, which are readily recognizable on sectioning of the gland: the cortex is radially striated and lighter in color than the medulla (Dyce et al., 2010). A fibrous capsule covers the cortex.

The abdominal aorta, renal artery, cranial abdominal artery and caudal phrenic artery send small branches to the adrenal glands (König and Liebich, 2009). The capillaries radiate from the cortex into the medulla and form a capsular and a medullary network. Venous blood is collected in the central vein and then passed through emissary vessels to the caudal vena cava. Lymphatic capillaries form a network within the adrenal parenchyma and drain into the lumbar aortic lymph nodes.

Aldosterone: synthesis, actions, metabolism and regulation

Aldosterone, or 4-pregnen-11β,21-diol-3,18,20-trione, is a steroid hormone with strong mineralocorticoid activity. It was historically considered to be a purely endocrine agent, produced exclusively in the adrenal cortex. However, recent research has revealed that aldosterone is also produced in tissues other than the adrenal cortex, including the heart, brain and blood vessels (for review, see Connell et al., 2008). In these extra-adrenal tissues aldosterone is thought to act in a paracrine or autocrine mode. Although these new insights contribute to the understanding of a number of long-term complications of primary hyperaldosteronism, and will be discussed briefly, this introduction will mainly focus on aldosterone as an endocrine agent.

Aldosterone is produced in the zona glomerulosa of the adrenal gland, the outermost zone of the cortex. Unlike cells of the other two zones – the middle zona fasciculata and the inner zona reticularis – glomerulosa cells contain the enzyme aldosterone synthase (CYP11B2), which catalyzes the final step in the conversion of cholesterol to aldosterone. Zona glomerulosa cells produce aldosterone “on demand”, for which purpose they contain cholesterol esters, mainly originating from circulating low-density lipoproteins. Upon stimulation of aldosterone production, cholesterol esters are rapidly converted to cholesterol and then, in five steps, to aldosterone (Figure 1), which is released into the circulation. As there is no specific binding globulin for aldosterone in the plasma, it is mainly bound with low affinity to albumin. This explains its relatively low circulating concentration in comparison with that of cortisol.

The epithelia of the kidneys, colon and salivary glands are the classic target tissues for circulating aldosterone. It readily passes the plasma membrane of these epithelial cells and binds to the cytoplasmic mineralocorticoid receptor. Although this receptor has equal affinity for aldosterone and cortisol, and the circulating concentration of cortisol is much higher than that of aldosterone, the mineralocorticoid receptor in the classic aldosterone target tissues is preferentially made available to aldosterone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). This enzyme converts cortisol to cortisone, which has little affinity for the receptor.

The aldosterone-receptor complex is translocated to the nucleus, where it modulates the expression of multiple genes. In epithelial cells of the distal nephron this ultimately results in activation of amiloride-sensitive sodium channels in the apical membrane.

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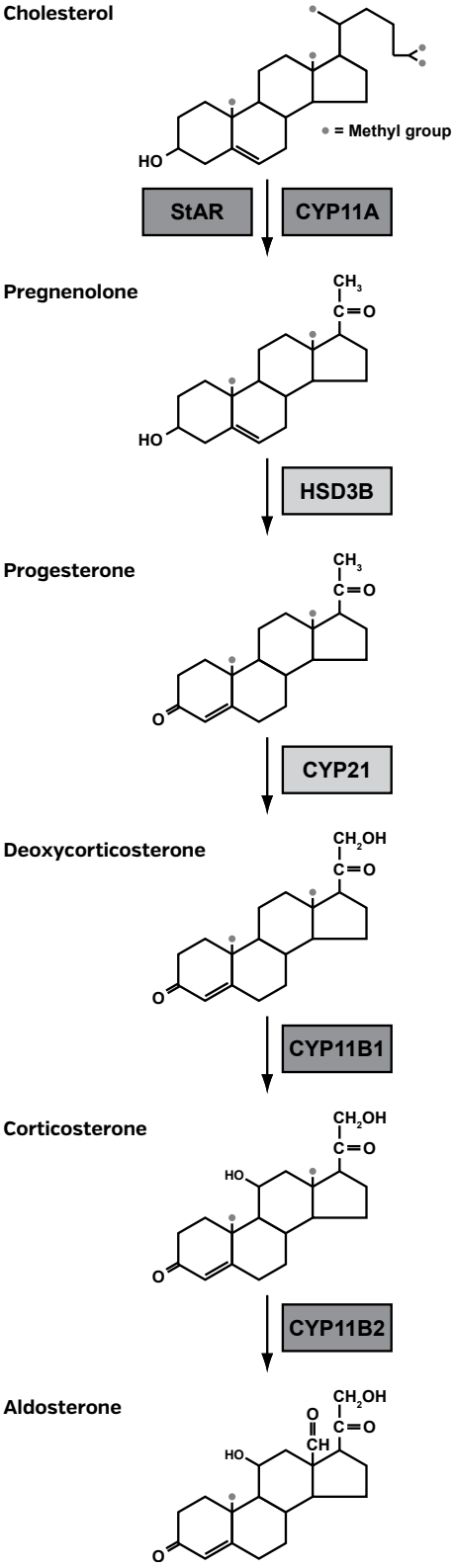

Figure 1
Aldosterone biosynthesis in the adrenal zona glomerulosa. The cellular location of the enzymes is indicated by the color of the text box:
light gray = smooth endoplasmic reticulum
dark gray = mitochondria
StAR = steroidogenic acute regulatory protein
CYP11A = cholesterol side chain cleavage enzyme
HSD3B = 3β-hydroxysteroid dehydrogenase
CYP21 = 21-hydroxylase
CYP11B1 = 11β-hydroxylase type 1
CYP11B2 = 11β-hydroxylase type 2 (aldosterone synthase)

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The resulting increased sodium influx stimulates the Na^+,K^+-ATPase in the basolateral membrane. The aldosterone-mediated increase in active sodium reabsorption from the urine generates an electrochemical gradient that facilitates the passive transfer of potassium from the tubular cells into the urine. Thus potassium is not excreted in direct exchange for sodium, but in a manner depending directly on the active reabsorption of sodium (for review, see Galac et al., 2010).

In addition to its endocrine effects on classic epithelial target tissues, aldosterone has major actions on other epithelial and non-epithelial tissues. Actions of aldosterone, probably in part nongenomic, on endothelial cells and on cardiac tissue contribute to blood pressure homeostasis (for review, see Connell et al., 2008). It appears that aldosterone may increase blood pressure through two main mechanisms: (1) expansion of plasma and extracellular fluid volume, and (2) increased total peripheral resistance. With regard to the non-epithelial actions, it should be added that long-term mineralocorticoid excess may lead to microangiopathies with fibrosis and proliferation of endothelial and smooth muscle cells, in tissues such as heart and kidney (Joffe et al., 2007).

Little is known about the metabolism of aldosterone in cats. The liver is generally considered to be the most important site for inactivation and conjugation of steroid hormones. In cats, cortisol, estradiol and progesterone are excreted mainly or almost exclusively via the bile into the feces (Brown et al., 1994; Graham and Brown, 1996), and, considering the structural similarities, it can be expected that this is also the main excretion route for aldosterone. This assumption is supported by a study of Syme and co-workers, who found that urinary excretion of free aldosterone in cats was 77 times less than in humans, and 7 times less than in dogs, accompanied by insignificant amounts of aldosterone-18-glucuronide (Syme et al., 2007).

The two primary regulators of aldosterone release are the renin-angiotensin system and potassium (Figure 2). The renin-angiotensin system maintains a constant circulating blood volume by promoting aldosterone-induced sodium retention during periods of hypovolemia and by decreasing aldosterone-dependent sodium retention during hypervolemia. Potassium ions directly regulate aldosterone secretion, independent of the renin-angiotensin system. Hyperkalemia stimulates aldosterone secretion by depolarizing (and hypokalemia inhibits it by repolarizing) the membranes of the zona glomerulosa cells (Aguilera and Catt, 1986). Thus aldosterone secretion is regulated via negative feedback loops for both potassium and the renin-angiotensin system. In addition to these two regulatory mechanisms, aldosterone secretion is influenced by several other factors (ACTH, natriuretic peptides, and a variety of neurotransmitters), none of which has direct or indirect negative feedback loops on aldosterone secretion.

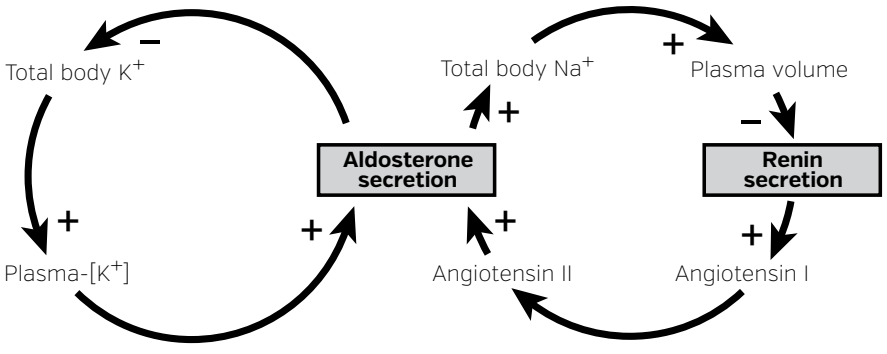

Figure 2
The regulation of aldosterone secretion in the zona glomerulosa of the adrenal cortex. The two main regulators are angiotensin II and potassium (K^+).

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The vast majority of the physiological actions of the renin-angiotensin system, such as vasoconstriction and aldosterone production, are mediated by angiotensin II and one of its receptors (angiotensin-II type 1 receptor, AT_1R). Angiotensinogen, mainly produced in the liver, is the precursor of several angiotensin peptides. In the circulation, angiotensinogen is cleaved by renin and other enzymes to release angiotensin I. The angiotensin-converting enzyme (ACE) converts the inactive decapeptide angiotensin I to the active octapeptide angiotensin II (Figure 2). ACE-inhibiting compounds are used clinically to disrupt the renin-angiotensin system, as in the treatment of heart failure.

The proteolytic enzyme renin is synthesized at a variety of locations, of which the juxtaglomerular cells of the kidney are the most well-known. Stimulation of renal baroreceptors is the most potent mechanism for its release. These stretch receptors in the afferent arteriole stimulate renin release in response to reduced renal perfusion pressure. Additional regulation is provided by the macula densa, a group of modified cells of the distal tubule near the end of the loop of Henle and intimately associated with the juxtaglomerular cells. Sodium concentration in the tubular lumen is monitored by the cells of the macula densa and low sodium levels trigger communication between the macula densa and the juxtaglomerular cells, resulting in renin release (for review, see Galac et al., 2010).

Pathophysiology of primary hyperaldosteronism

Primary hyperaldosteronism, also referred to as primary aldosteronism or Conn's syndrome, is an adrenocortical disorder characterized by excessive, autonomous secretion of mineralocorticoids, mainly aldosterone, leading to systemic arterial hypertension and/or hypokalemia (Galac et al., 2010). The autonomous hypersecretion of aldosterone originates from neoplastic or hyperplastic zona glomerulosa tissue and is associated with suppressed plasma renin activity. Hence the condition is also designated as low-renin hyperaldosteronism, as opposed to high-renin or secondary hyperaldosteronism, which is a pathophysiological response to hypovolemia. In secondary hyperaldosteronism, a reduction in the effective arterial blood volume, as due to heart failure or edema caused by hypoproteinemia, activates the renin-angiotensin system, which in turn persistently stimulates aldosterone synthesis. Therefore, by definition, the excessive aldosterone production in secondary hyperaldosteronism is not autonomous.

The pathophysiological consequences of excessive aldosterone secretion are related to increased sodium and water retention and increased renal potassium excretion, resulting in systemic arterial hypertension and potassium depletion, respectively. The progressive depletion of potassium and the development of hypokalemia affect several organ systems, but become particularly manifest in the neuromuscular system by affecting the polarization of nerve and muscle membranes leading to muscle weakness.

Primary hyperaldosteronism, especially when due to micronodular hyperplasia of the zona glomerulosa, has been associated with cardiovascular and renal complications in humans (Connell et al., 2008). It has been hypothesized that the mild hyperaldosteronism with incomplete renin suppression associated with micronodular hyperplasia of the zona glomerulosa results in the combined deleterious, proinflammatory and profibrotic effects of elevated aldosterone and angiotensin II levels. Whether this also plays a role in the pathophysiology of feline hyperaldosteronism remains to be determined.

Primary hyperaldosteronism in man

Primary hyperaldosteronism or Conn's syndrome was first described in 1955, in a woman presenting with arterial hypertension, severe hypokalemia and alkalosis

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(Conn, 1955). Conn suggested that as many as 20% of people with arterial hypertension would have primary hyperaldosteronism (Conn, 1967). Nevertheless, primary hyperaldosteronism was considered a very rare condition for many decades. With improved screening tests, however, detection has increased and recent studies have shown that the prevalence of primary hyperaldosteronism is quite high indeed: it is found in about 6% of all human patients with arterial hypertension and up to 11% of those selected for therapy-resistant hypertension (Fogari et al., 2007; Douma et al., 2008).

The most common cause of primary hyperaldosteronism in humans is bilateral nodular hyperplasia of the zona glomerulosa (60-65%), followed by aldosterone-producing adenomas (30-35%) (Young, 2007). Unilateral adrenocortical hyperplasia is uncommon, and glucocorticoid-remediable aldosteronism and functional adrenocortical carcinomas are rare.

Primary hyperaldosteronism is suspected in patients with moderate, severe or resistant arterial hypertension, spontaneous or diuretic-induced hypokalemia, arterial hypertension combined with an adrenal incidentaloma, or a family history of early-onset hypertension or cerebrovascular accident at an age of 40 years or younger (Funder et al., 2008). In these patients the ratio of plasma aldosterone concentration (PAC) to plasma renin activity (PRA) (the aldosterone-to-renin ratio, ARR) is used to uncover inappropriate aldosterone secretion (Hiramatsu et al., 1981). Since renin is not the sole regulator of aldosterone release, false-positive and false-negative test results are possible, and therefore the ARR is regarded as a screening test only in human medicine. For an accurate interpretation of test results, endogenous factors that influence the ARR should be corrected and exogenous factors avoided where feasible (for review, see Stowasser et al., 2012). Consequently, the patient should be potassium-replete, on an unrestricted dietary salt intake, and four weeks' off potassium-wasting diuretics or spironolactone at the time of sampling (Funder et al., 2008).

A positive screening test should be followed by confirmative testing (Funder et al., 2008). Aldosterone suppression tests have been designed to demonstrate the autonomy of aldosterone hypersecretion in humans. The exogenous substance administered for these tests interacts with the renin-angiotensin system feedback loop and thereby, under physiological circumstances, inhibits aldosterone secretion. A lack of aldosterone suppression confirms the autonomy of aldosterone hypersecretion. Tests include (1) the oral or intravenous sodium loading test (i.e. responsiveness to plasma volume expansion); (2) the captopril suppression test (i.e. responsiveness to reduced angiotensin II levels); and (3) the fludrocortisone suppression test (i.e. responsiveness to mineralocorticoid-induced plasma volume expansion). Before any confirmative testing is commenced it is important to discontinue mineralocorticoid receptor blockers and diuretic medications, in order to avoid unintentional interaction with the renin-angiotensin-aldosterone system.

Adrenal vein sampling was introduced in human medicine in the late-1960s and, despite potentially severe complications, it has become the gold standard to determine the laterality (left or right adrenal) of excessive aldosterone production in humans (Melby et al., 1967). Each adrenal vein is cannulated in turn and samples are collected while peripheral venous blood samples are collected simultaneously (Daunt, 2005). The plasma aldosterone and cortisol concentrations in the adrenal and peripheral venous samples are compared to detect the source of excess aldosterone.

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Primary hyperaldosteronism in cats

As in human medicine, the knowledge of primary hyperaldosteronism in feline medicine started with a single case report (Eger et al., 1983). Within the last two decades of the second millennium only a few case descriptions appeared in veterinary literature (Flood et al., 1999; MacKay et al., 1999), and primary hyperaldosteronism was regarded a rare disease in cats. However, the number of case reports has risen considerably in the first decade of the current century (for review, see Djajadiningrat-Laanen et al., 2011). It is to be expected that, as in man, increasing awareness of the disease will result in a further increase in numbers of diagnosed and reported cases.

Primary hyperaldosteronism is probably the most common adrenocortical disorder in cats and it may be an important cause of arterial hypertension in this species, as it is in man. Although the cat is considered to be the domestic animal in which primary hyperaldosteronism is most prevalent, the disease is not often diagnosed in veterinary practice. It is most likely underdiagnosed, as it is in humans, which excludes a potentially large number of cats from appropriate therapy and possibly a cure for the disease. This may in part be due to the frequent association of arterial hypertension and/or hypokalemia with chronic renal disease. In many cases of arterial hypertension and/or hypokalemia, chronic renal disease may be considered the causal disorder, thereby halting further diagnostic efforts – whereas in fact the chronic renal failure itself might be a consequence of primary hyperaldosteronism, as has been demonstrated in humans (for review, see Connell et al., 2008). Furthermore, arterial hypertension and hypokalemia are often treated symptomatically only, without a thorough search for the underlying cause. Moreover, arterial blood pressure is not measured routinely, if at all, in many veterinary practices.

Mineralocorticoid excess in cats mainly occurs in middle and old age. It is caused by either idiopathic bilateral nodular hyperplasia of the zona glomerulosa (Figure 3), or by unilateral or bilateral neoplasia of the zona glomerulosa (Figure 4). Histopathological findings in 33 cats with primary hyperaldosteronism suggest that unilateral adrenocortical carcinoma (15 reported cases) and unilateral adrenocortical adenoma (11 cases) occur with similar frequency, whereas bilateral adenoma (2 cases), unilateral carcinoma and contralateral adenoma (1 case), and bilateral nodular hyperplasia (4 cases) seem to occur less frequently (Eger et al., 1983; Flood et al., 1999; MacKay et al., 1999; Bruyette, 2001; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005; Reimer et al., 2005; Rose et al., 2007; Renschler and Dean, 2009; Smith et al., 2012; Willi et al., 2012). These reported figures appear to differ markedly from those in humans, where bilateral hyperplasia of the zona glomerulosa is the most common etiology of primary hyperaldosteronism (Young, 2007). However, the diagnosis of idiopathic primary hyperaldosteronism in humans can be established clinically, whereas in cats histopathological examination of the adrenal glands is required – and because cats with hyperplasia of the zona glomerulosa are often treated medically, adrenal tissue is not examined histologically except in post-mortem examinations. This probably means that idiopathic bilateral nodular hyperplasia of the zona glomerulosa occurs more often in cats than suggested by data based on histopathological findings.

Presenting signs of primary hyperaldosteronism in cats

About 49 cases of presumed or confirmed feline primary hyperaldosteronism have been reported (Eger et al., 1983; Flood et al., 1999; MacKay et al., 1999; Maggio et al., 2000; Moore et al., 2000; Bruyette, 2001; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005;

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Javadi et al., 2005; Reimer et al., 2005; Rose et al., 2007; Briscoe et al., 2009; Renschler and Dean, 2009; Smith et al., 2012; Willi et al., 2012). Affected cats were presented at a median age of 13 years (range 5-20 years; n=42; in the remaining cases the age was not specified). There has been no apparent sex predilection and the breeds have included domestic shorthair (30 cats), domestic longhair (4 cats), British shorthair (2 cats), Siamese (2 cats), Persian (2 cats), and Burmese, Burmilla and Tonkinese (1 each); the breed was not specified in 6 cats.

Clinical signs comprise signs related to muscular weakness and to ocular complications of arterial hypertension. Muscle weakness is likely to occur at plasma potassium concentrations around 2.5 mmol/L, although the severity of muscle weakness is not strictly correlated with the plasma potassium concentration. Signs of muscle

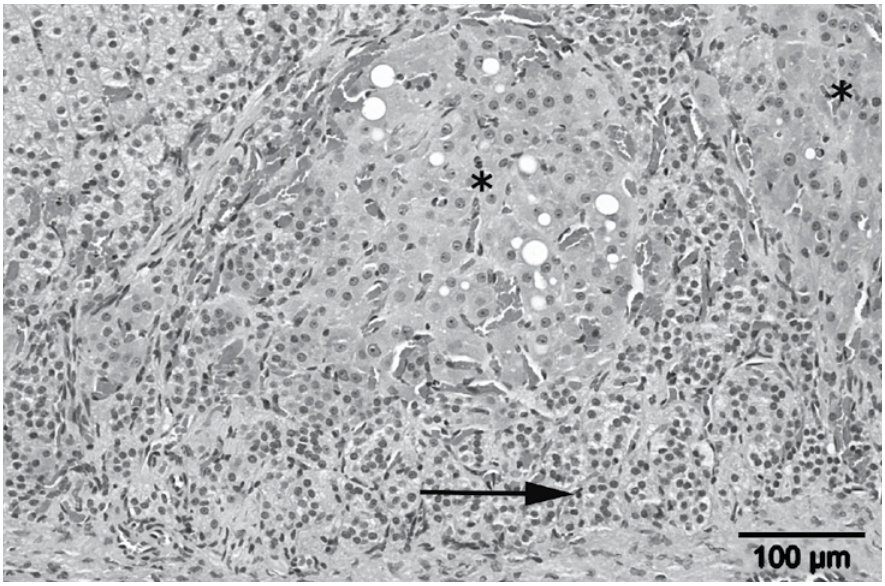

Figure 3
Adrenal of a hypokalemic cat with multiple cortical hyperplastic nodules (asterisks). Note the pre-existing zona glomerulosa of the adrenal gland (arrow). Haematoxylin and eosin stain.

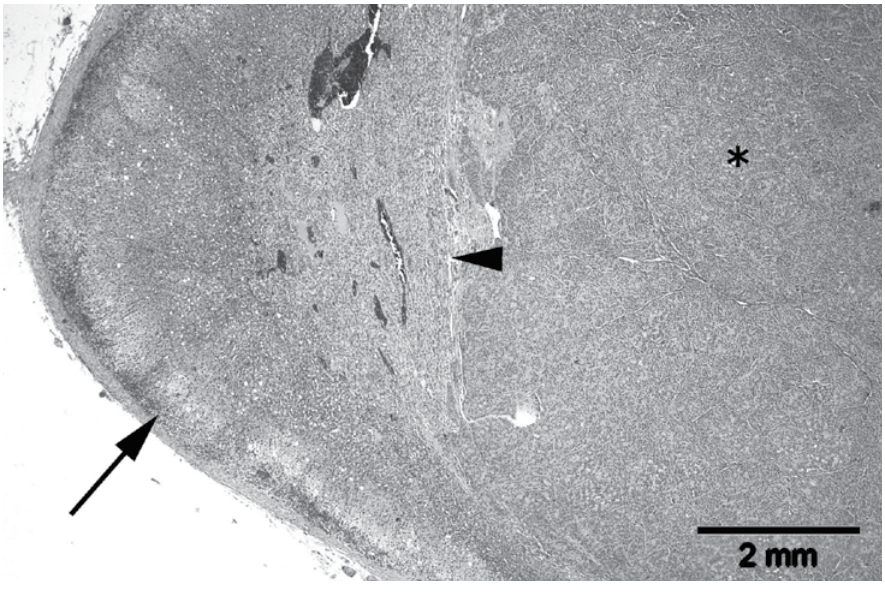

Figure 4
Histological section of a neoplasm (asterisk) of the adrenal cortex in a cat with primary hyperaldosteronism. Note the compression of the pre-existing adrenal medulla (arrowhead). There is no marked atrophy in the uncompressed areas of the pre-existing adrenal cortex (arrow). Haematoxylin and eosin stain.

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weakness are sometimes preceded by dysphagia or episodic forelimb stiffness, and include episodic or acute generalized weakness, a plantigrade stance of the hind limbs, difficulty in jumping and/or a characteristic ventroflexion of the neck; in some cases there is progression to flaccid paresis with hyporeflexia, muscle hypotonia and difficulty in breathing (Figure 5).

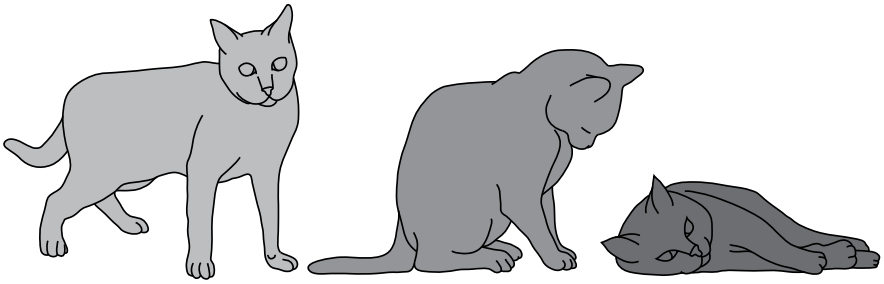

Figure 5
Three of the clinical manifestations of muscle weakness in cats with hypokalemia. From left to right: plantigrade stance, cervical ventroflexion, lateral recumbency due to flaccid paresis.

Ocular complications of arterial hypertension cause the most striking and thus usually the presenting signs of arterial hypertension. They include transient anisocoria, mydriasis, hyphema and loss of vision due to retinal detachment and/or intraocular hemorrhages.

Not all cats with primary hyperaldosteronism present with signs of hypokalemia or arterial hypertension. Some present with a pendulous abdomen; with polyuria, nocturia and polydipsia; or rather non-specific signs such as anorexia, weight loss, depression, restlessness or panting.

Physical and laboratory findings in cats with primary hyperaldosteronism

Abnormal physical findings have been related mainly to systemic arterial hypertension and potassium depletion, including elevated arterial blood pressure (35 of 44 cats in which it was measured); hypertensive ocular signs including mydriasis, hyphema, increased tortuosity of the retinal vessels, retinal edema and loss of vision due to retinal detachment and/or intraocular hemorrhages (16 of 25 cats in which an ophthalmic examination was performed); and hypokalemic polymyopathy (27 cats). Other findings included a palpable mass in the cranial abdomen (3 cats), pronounced muscle atrophy, cutaneous fragility in two cases of combined hyperaldosteronism and hyperprogesteronism, a systolic heart murmur and an irregular cardiac rhythm. Congestive heart failure was diagnosed in one cat with combined primary hyperaldosteronism and hyperprogesteronism. In two cases no abnormalities were found by the initial clinical examination.

Abnormalities found in routine laboratory examinations included hypokalemia (43/49 cats), elevated values for plasma urea (19/37 cats), creatinine (17/34 cats) and creatinine kinase activity (19/20 cats), hyperglycemia (4 cats), hypomagnesemia (2 cats), hypochloremia (3 cats), hypophosphatemia (3/13 cats) and hyperphosphatemia (1/13 cats). Among all 37 hypertensive cats, 32 were concurrently hypokalemic and 4 were normokalemic, although in one cat plasma potassium concentration was at the lower end of the reference range. Hypernatremia, combined with hypokalemia, was found in only 3 of the 38 cats in which the plasma sodium concentration was measured. Plasma progesterone concentration was elevated in 2 cats and was associated with diabetes mellitus in both.

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Plasma aldosterone concentration was increased in 39 of 49 reported cases and plasma renin activity was below or within the reference ranges in all 23 cats in which it was measured. Although plasma aldosterone concentration was within the reference range in the remaining 10 of the 49 cats, and even plasma renin activity was within the reference range in 2 of these, the aldosterone-to-renin ratio was elevated in all 7 cats in which plasma renin activity was measured.

Diagnostic investigation of cats suspected of primary hyperaldosteronism
Primary hyperaldosteronism should be considered in any cat found to have an elevated arterial blood pressure and/or hypokalemia; in particular if hypertension and/or hypokalemia are relatively refractory to treatment. Indirect measurement of arterial pressure should be performed by a Doppler or oscillometric technique, according to the ACVIM consensus statement on arterial hypertension in dogs and cats (Brown et al., 2007). Routine laboratory investigation ideally includes urinalysis and measurement of plasma concentrations of sodium, potassium, urea, creatinine, glucose, fructosamine, calcium, phosphate and thyroxine.

If, based on history, physical examination and laboratory results, primary hyperaldosteronism is considered likely, a screening test for abnormal regulation of aldosterone production should be performed. If regulation is abnormal, the diagnostic investigation should ideally include a test to confirm the diagnosis, diagnostic imaging of the adrenal glands and of predilection sites for metastases, and determination of whether the left or the right adrenal is the site of abnormal aldosterone production. The latter two procedures are necessary for planning treatment, since unilateral primary hyperaldosteronism due to an adrenocortical adenoma or adenocarcinoma can potentially be cured surgically, whereas a metastasized adrenocortical adenocarcinoma or bilateral primary hyperaldosteronism due to hyperplasia of the zona glomerulosa should be controlled medically.

Screening for primary hyperaldosteronism in cats
As in humans, the ratio of plasma aldosterone concentration (PAC) to plasma renin activity (PRA) (the aldosterone-to-renin ratio, ARR) is used to screen for abnormal regulation of aldosterone production in cats (Javadi et al., 2004; Javadi et al., 2005). In cats with unilateral or bilateral zona glomerulosa tumors, the PAC may be very high and PRA is usually completely suppressed (Eger et al., 1983; Flood et al., 1999; Moore et al., 2000; Bruyette, 2001; Rijnberk et al., 2001; Briscoe et al., 2009; Smith et al., 2012). In cats with idiopathic bilateral nodular hyperplasia of the zona glomerulosa, the PAC may be only slightly elevated or within the upper limit of the reference range (Javadi et al., 2005). Since hypokalemia is the predominant factor lowering the PAC, the plasma potassium concentration should be considered in evaluating the PAC. In the presence of hypokalemia, even a mildly elevated aldosterone level can be regarded as inappropriately high. The PRA must also be taken into account. The combination of a high-normal or elevated PAC and a low PRA indicates persistent aldosterone synthesis in the presence of little or no stimulation by the renin-angiotensin system.

The diagnostic value of the ARR is mainly determined by the sensitivity of the renin assay. In primary hyperaldosteronism PRA is low, and therefore a relatively high renin detection limit can have a profound effect on the ARR (Montori and Young, 2002; Young, 2002). Furthermore, PRA values should be interpreted in comparison with an appropriate control population. The accuracy of the ARR also depends on preservation of

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renin activity during sample collection and storage: blood samples should be collected in ice-chilled tubes and centrifuged in a chilled centrifuge, and the plasma should be frozen immediately and kept frozen until assayed.

Although the ARR is currently the gold standard for screening for feline primary hyperaldosteronism, it has some disadvantages. These include the necessity for a large (4 mL) blood sample and instant freezing of the separated plasma. Furthermore, PRA measurements are time-consuming, and reference values for PRA may differ markedly between laboratories, making comparison difficult. Finally, as in human medicine, repeated sampling for the ARR may be required (Stowasser et al., 2012).

An alternative means of diagnosis might be the measurement of the urinary aldosterone-to-creatinine ratio (UACR). As opposed to the ARR, which discloses the aldosterone secretion at a single moment in time, the UACR provides an integrated reflection of aldosterone secretion over time. As an additional advantage, a urine sample for measurement of aldosterone can be collected quite easily and does not have to be frozen immediately. Before the UACR can be used in the diagnostic approach of a cat suspected of primary hyperaldosteronism, reference values have to be established. Next, it has to be determined whether the UACR reliably differentiates between cats with and without primary hyperaldosteronism.

Confirmatory tests for primary hyperaldosteronism in cats

Unfortunately, no validated test is available to confirm primary hyperaldosteronism in cats. A test employing a suppressive agent that reduces aldosterone secretion in healthy cats but has little or no effect in those with primary hyperaldosteronism would seem to be the best means of showing the presence of a hyperfunction of zona glomerulosa tissue.

Diagnostic imaging

Diagnostic imaging techniques such as ultrasonography (Figure 6), magnetic resonance imaging (MRI), and computed tomography (CT) are used to identify adrenal abnormalities and, in case of neoplasia, to evaluate possible extension into the caudal vena cava and the presence of distant metastases. Although the presence of visible tumor

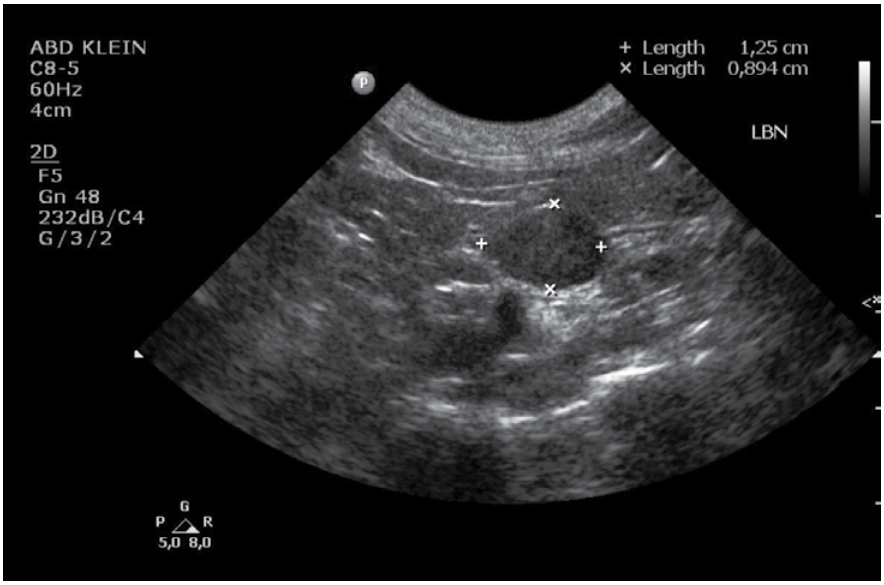

Figure 6
Ultrasonographic image of the left adrenal gland of a cat with primary hyperaldosteronism due to a unilateral adrenocortical adenoma. The gland appears to be enlarged and hyperechogenic.

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tissue in the caudal vena cava indicates that surgical removal may be difficult, failure to detect it by diagnostic imaging is no guarantee of its absence and does not necessarily predict an uncomplicated adrenalectomy (Ash et al., 2005; DeClue et al., 2005).

There are more limitations to conventional diagnostic imaging in determining the optimal treatment strategy for primary hyperaldosteronism. Functional neoplasms of the zona glomerulosa need not be large in order to cause clinically relevant hyperaldosteronism, and may therefore be well below the detection limit of ultrasonography, CT, or MRI. Similarly, clinically relevant hyperplasia of the zona glomerulosa may not be revealed by conventional diagnostic imaging techniques. On the other hand, non-functional adrenocortical neoplasms may grow to considerable proportions and be readily visualized with ultrasonography, CT, or MRI, but may not cause clinical signs. Therefore, a visible adrenal mass may not be a functional neoplasm of the zona glomerulosa which is causing the clinical signs of primary hyperaldosteronism, and if surgery is planned on the basis of conventional diagnostic imaging alone, the wrong adrenal gland may be removed or the patient may be inappropriately selected for or excluded from adrenalectomy. Comparing the results of morphological diagnostic imaging techniques (CT and MRI) with adrenal venous sampling in human patients with primary hyperaldosteronism, Kempers and co-workers concluded that CT/MRI results did not accurately identify the source of aldosterone excess in as many as 38% of 950 patients (Kempers et al., 2009). Similarly, adrenal diagnostic imaging results appeared inaccurate in 5 of 30 cats with histopathologically confirmed primary hyperaldosteronism (Flood et al., 1999; MacKay et al., 1999; Bruyette, 2001; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005; Reimer et al., 2005; Rose et al., 2007; Renscher and Dean, 2009; Smith et al., 2012; Willi et al., 2012). One cat in which ultrasonography revealed a right adrenal mass and a normal left adrenal gland was found by post-mortem examination 13 days later to have bilateral adrenocortical adenoma (Ash et al., 2005). The left adrenal of another cat was removed because ultrasonography and CT indicated asymmetrical thickening, but bilateral nodular hyperplasia of the zona glomerulosa was found (Javadi et al., 2005). In 3 other cats, ultrasonography (3 cats) and CT (2 cats) revealed no abnormality of the adrenals, but nodular hyperplasia was confirmed by histological examination (Javadi et al., 2005; Willi et al., 2012). Distant metastases of adrenocortical adenocarcinomas may be missed on diagnostic images if their size is below the detection limit of the technique. This has been documented in one case, in which thoracic radiography failed to reveal pulmonary metastases 3 mm in diameter (Rijnberk et al., 2001).

Nuclear medicine imaging of the adrenal glands is a relatively new technique in human endocrinology. Both ^{11}C-metomidate positron emission tomography (PET) scanning and ^{131}I-6β-iodomethyl-19-norcholesterol single photon emission computed tomography (SPECT) have proved useful (Eriksson et al., 2005; Yen et al., 2009). It is expected that these techniques will eventually become available in veterinary medicine and will prove to be valuable in the diagnosis of feline primary hyperaldosteronism.

Determining the laterality of hyperaldosteronism

Adrenal vein sampling has become the gold standard to determine the laterality (left or right adrenal) of excessive aldosterone production in humans (Melby et al., 1967). Unfortunately, the much smaller vascular dimensions in cats preclude adrenal venous sampling and thus determination of the laterality of primary hyperaldosteronism continues to rely on diagnostic imaging.

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Treatment and prognosis of feline primary hyperaldosteronism

Unilateral adrenalectomy is the treatment of choice for confirmed unilateral primary hyperaldosteronism. There have been several reports of successful surgical intervention (Flood et al., 1999; MacKay et al., 1999; Ash et al., 2005; Reimer et al., 2005; Renschler and Dean, 2009; Smith et al., 2012; Willi et al., 2012), including the excision of an adrenocortical carcinoma together with its extension into the vena cava (Rose et al., 2007). Preoperatively and perioperatively, hypokalemia should be controlled as well as possible by oral and intravenous supplementation. During the first few weeks after surgery a generous dietary intake of sodium can be provided to avoid hyperkalemia resulting from the chronic suppression of aldosterone secretion in the contralateral adrenal. In humans with primary hyperaldosteronism, preoperative spironolactone administration for at least 1-2 months may activate the suppressed renin-angiotensin system, and thereby aldosterone production in the contralateral, suppressed adrenal gland (Don et al., 1997). This might be beneficial in cats as well. Temporary postoperative administration of fludrocortisone could also be considered, but was not found necessary in the cases that have been reported. After complete removal of a unilateral non-metastasized mineralocorticoid-producing tumor, the prognosis is excellent, with no medication in most cases. Most of the cats that survived the immediate postoperative period have continued to be clinically asymptomatic for one to several years. However, perioperative complications were also reported in 8 of 24 surgical cases (MacKay et al., 1999; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005). They included intraoperative or postoperative intra-abdominal hemorrhage (6 cases), acute renal failure (1), sepsis (1) and suspected thromboembolism (1), and in 6 cases the outcome was fatal. Furthermore, arterial hypotension and hypoglycemia occurred in a cat recovering from unilateral adrenalectomy and venous thrombectomy under deliberate hypothermia. Whereas postoperative sepsis and thromboembolism have also been described in cats following adrenalectomy for other primary conditions, intra- and postoperative hemorrhages have been reported more frequently in association with adrenalectomy for primary hyperaldosteronism (MacKay et al., 1999; Rijnberk et al., 2001; Ash et al., 2005). Risk factors have not yet been identified. Hemorrhage was not specifically related to type of neoplasia, intravenous tumor extension, or the presence or absence of arterial hypertension as a presenting clinical sign. Therefore, all owners considering surgical management of primary hyperaldosteronism in their cat should be informed of this potential complication.

If surgery is precluded because of bilateral zona glomerulosa hyperfunction, a non-resectable unilateral adrenocortical neoplasm, distant metastases, financial limitations, or comorbid conditions, medical therapy with a mineralocorticoid receptor blocker is instituted, together with potassium supplementation and antihypertensive drugs if needed. The aldosterone receptor blocker most often used in cats is spironolactone. The initial dose is 2 mg per kg body weight orally, twice daily, increased as needed to control hypokalemia. A dose in excess of 4 mg/kg may cause anorexia, diarrhea and vomiting. In cats, hyperaldosteronism due to bilateral adrenocortical hyperplasia is usually somewhat milder than that due to neoplasia, and normokalemia may be sustained for long intervals with spironolactone alone or combined with low doses of potassium (Javadi et al., 2005). Persistent arterial hypertension can be treated with the calcium blocker amlodipine, at an initial oral dose of 0.1 mg per kg body weight, once daily.

Although some cats with medically managed primary hyperaldosteronism have been reported to live for up to several years (Ash et al., 2005; Javadi et al., 2005), the prognosis may not be as favorable as that after complete removal of an aldosterone-producing neoplasm, for medical treatment does not abolish the mineralocorticoid excess as definitely as surgery may do.

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03

Primary hyperaldosteronism, a mediator of progressive renal disease in cats

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Abstract

In recent years, there has been renewed interest in primary hyperaldosteronism, particularly because of its possible role in the progression of kidney disease. While most studies have concerned humans and experimental animal models, here we report on the occurrence of a spontaneous form of (non-tumorous) primary hyperaldosteronism in cats. At presentation, the main physical features of 11 elderly cats were hypokalemic paroxysmal flaccid paresis and loss of vision due to retinal detachment with hemorrhages. Primary hyperaldosteronism was diagnosed on the basis of plasma concentrations of aldosterone (PAC) and plasma renin activity (PRA), and the calculation of the aldosterone-to-renin ratio (ARR). In all animals, PACs were at the upper end or higher than the reference range. The PRAs were at the lower end of the reference range, and the ARRs exceeded the reference range. Diagnostic imaging by ultrasonography and computed tomography revealed no or only very minor changes in the adrenals compatible with nodular hyperplasia. Adrenal gland histopathology revealed extensive micronodular hyperplasia extending from zona glomerulosa into the zona fasciculata and reticularis. In three cats, plasma urea and creatinine concentrations were normal when hyperaldosteronism was diagnosed but thereafter increased to above the upper limit of the respective reference range. In the other eight cats, urea and creatinine concentrations were raised at first examination and gradually further increased. Even in end-stage renal insufficiency, there was a tendency to hypophosphatemia rather than to hyperphosphatemia. The histopathological changes in the kidneys mimicked those of humans with hyperaldosteronism: hyaline arteriolar sclerosis, glomerular sclerosis, tubular atrophy and interstitial fibrosis. The non-tumorous form of primary hyperaldosteronism in cats has many similarities with “idiopathic” primary hyperaldosteronism in humans. The condition is associated with progressive renal disease, which may in part be due to the often incompletely suppressed plasma renin activity.

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Introduction

Injury of the glomeruli and the tubulointerstitium may initiate the cascade of pathogenetic events leading to chronic renal insufficiency. Excessive accumulation of extracellular matrix (ECM) plays a central role in this progressive loss of kidney function. Several mediators promote ECM accumulation, including growth factors such as transforming growth factor-β and connective tissue growth factor (Fogo, 2000). In addition, the renin-angiotensin-aldosterone system has been implicated in progressive renal sclerosis.

The hemodynamic and non-hemodynamic actions of angiotensin II were initially thought to be responsible for the progression of renal insufficiency. Angiotensin II is not only a secretagogue for aldosterone, a peripheral vasoconstrictor and a regulator of glomerular filtration, but also a growth factor and a true cytokine (Fogo, 2000; Wolf, 2001). It may act as a growth factor regulating hyperplasia or hypertrophy of mesangial, glomerular endothelial and tubuloepithelial cells, as well as renal interstitial fibroblasts. In addition, there is increasing evidence that angiotensin II is involved in the regulation of inflammatory and immune-cell responses, and thus may have an active role in the recruitment of inflammatory cells into the kidney. Angiotensin II is now considered a true proinflammatory modulator contributing to the onset and progression of kidney damage (Ruiz-Ortega et al., 2001).

However, recent evidence indicates that not only angiotensin II but also aldosterone per se may contribute to the progression of kidney damage by promoting thrombosis and fibrosis. Circulating aldosterone may mediate vascular fibrosis by interacting directly with high-affinity, low-capacity corticoid receptors located in the cytosol of vascular fibroblasts or by affecting the vascular fibrinolytic balance, i.e. the plasminogen activator system (Epstein, 2001). The current view is that both aldosterone and angiotensin II are instrumental in sustaining systemic arterial hypertension and fibroproliferative destruction of the kidney (Fogo, 2000; Hostetter et al., 2001; Feria et al., 2003).

Awareness of the pathophysiological role of aldosterone in renal disease prompts an interest in feline pathophysiology. Chronic renal insufficiency is relatively common in cats and is associated with systemic arterial hypertension (Syme et al., 2002). Although renal failure is often associated with hypokalemia (Dow et al., 1989; Polzin et al., 2000), the role of the renin-angiotensin-aldosterone system has not been elucidated. While one study has demonstrated plasma aldosterone concentration (PAC) and plasma renin activity (PRA) not to be significantly different from control values in cats with hypokalemia (Dow et al., 1987), two other studies of cats with renal insufficiency reported PACs to be higher than in control cats. In one of these studies, the increased PACs were associated with variable PRA values (Jensen et al., 1997), whereas in the other study, PRA was reported to be higher than in control cats (Mishina et al., 1998).

Here we report on cats with hyporeninemic hyperaldosteronism due to primary non-tumorous hyperaldosteronism, in which hyperaldosteronism was considered to mediate renal failure.

Case histories

Eleven cats (Table 1) were referred for endocrine consultation for various reasons: normal check-up (cat 2), hypokalemic paroxysmal flaccid paresis (cats 1, 4, 7), and retinal detachment and sub- and intraretinal and intravitreal hemorrhages associated with arterial hypertension (cats 3, 5, 6, 8-11). The case histories of two of these cats are presented as examples.

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Cat 1

This 13-year-old castrated female shorthaired cat was presented in an emergency situation because it had fallen off the refrigerator and had difficulty in walking. At presentation, the cat had a floundering gait and muscle weakness. It seemed to be blind. On physical examination, anisocoria was noted, the left pupil being more dilated that the right pupil. Ophthalmic examination revealed retinal detachment in both eyes, which was complete in the left eye and focal in the right eye. Systolic arterial blood pressure in the radial artery, measured by an indirect method (ultrasonic Doppler flow detector, cuff width 2.5 cm) was higher (200 mmHg = 26.6 kPa) than the reported upper limit of the reference range (Sparkes et al., 1999). The cat was hospitalized for correction of the hypokalemia (Table 1). Two days later, the cat's condition had improved, and it was discharged with home medication consisting of oral potassium supplements (twice daily 2 mmol KCl, Tumil-K®, Aesculaap, Boxtel, The Netherlands). The owner agreed that the cat would be recalled for adrenocortical function studies.

Cat 2

This 16-year-old female castrated Tonkinese cat was one of the cats used to establish the reference range for PRA and PAC (Rijnberk et al., 2001). The owner considered this cat to be healthy and in good condition, bearing in mind its age. The only reported abnormality was a transient anisocoria, which had occurred three months earlier and for which no cause had been identified. Ophthalmic examination had not revealed abnormalities and serology for possible causes such as feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline infectious peritonitis (FIP) had been negative. At the time of blood collection, the plasma concentrations of urea, creatinine, Na and K were within their respective reference ranges. However, PAC was elevated and the PRA values were immeasurably low, which are compatible with primary hyperaldosteronism (Table 2).

The cat was excluded from the reference population and the owner agreed to have the cat re-examined. At 67 days after the first blood collection, the routine blood biochemistry was reassuring (Figure 1) and it was decided not to take further measures. However, on day 253, the cat was brought in with a 3-day history of transient unilateral hyphema. On ophthalmoscope examination, retinal edema and multiple well-defined, small, circular to irregular areas of serous retinal detachment were seen in both eyes. Systolic blood pressure was 220 mmHg (= 29.3 kPa). The plasma creatinine concentration exceeded the reference range. Later on, hypokalemia also developed (Figure 1).

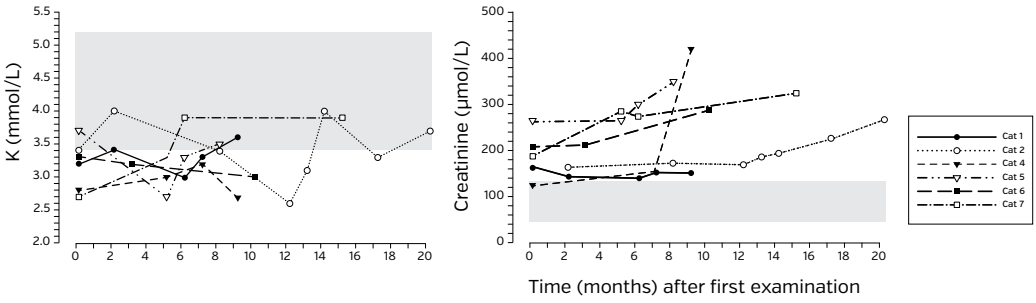

Figure 1

Changes in plasma concentrations of creatinine and potassium (K) with time in cats with non-tumorous (idiopathic) primary hyperaldosteronism. Plasma creatinine concentrations gradually increase, whereas the plasma potassium concentrations remain practically unchanged. Reference ranges are depicted by shaded areas.

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Cat no.	Breed	Age	Sex	Urea	Creatinine	Na	K	Ca	PO4	Thyroxine	SABP
		years		mmol/L	µmol/L	mmol/L	mmol/L	mmol/L	mmol/L	nmol/L	mmHg
1	D. Shorthair	13	FC	18.1	167	153	2.3	2.9	1.1	32	200
2	Tonkinese	16	FC	8.4	165	151	3.4	2.5	1.0	26	220
3	D. Shorthair	18	FC	13.1	222	154	3.6			26	190
4	D. Shorthair	15	MC	6.2	125	155	2.8	2.4	1.2	31	
5	B. Shorthair	15	MC	21.1	268	156	3.7	2.7	0.9	24	195
6	D. Shorthair	15	MC	10.8	209	156	3.3	2.8	0.9	36	240
7	D. Shorthair	14	FC	12.7	188	149	2.7	2.5	0.9	34	185
8	Persian	14	F	15.7	182	152	3.5			37	220
9	D. Shorthair	15	FC	12.6	241	153	3.1	3.2	1.3	22	270
10	D. Shorthair	12	FC	13.9	191	154	2.9	2.9	1.4	33	190
11	B. Shorthair	11	FC	7.2	101	151	3.6	2.3	1.6	11	230
Reference range		Lower limit		5.9	76	146	3.4	2.4	0.9	15	
		Upper limit		12.9	166	158	5.2	2.8	2.1	45	195

Table 1
Signalment and routine measurements in 11 cats with primary aldosteronism. B. Shorthair = British shorthair D. Shorthair = domestic shorthair FC = Female castrated MC = Male castrated SABP = Systolic arterial blood pressure

Cat no.	Days	Corticoid-to-creatinine ratio ($x10^{-6}$)		PAC	PRA	ARR
	> 1st ex	Day 1	Day 2	pmol/L	fmol/L/s	
1	2			130	40	3.3
	2			170	40	4.3
	13	7.4	7.7	370	40	9.3
2	0			830	<20	>41.5
	67			960	30	32.0
	253			780	45	17.3
	360			600	50	12.0
	410			1140	40	28.5
	437			1670	50	33.4
3	0			750	70	10.7
4	0			280	70	4.0
	20	13	14	490	60	8.2
	151			505	65	7.8
	213			375	70	5.4
5	0			950	80	11.9
	0	2.4	2.2	870	110	7.9
	166			670	160	4.2
6	0			440	50	8.8
	88			415	235	1.8
	293			540	220	2.5
7	165			750	110	6.8
8	462			290	10	29.0
9	6			530	120	4.4
10	0			280	10	28
11	0			390	30	13
Reference range	Lower limit	2		110	60	0.3
	Upper limit	36		540	630	3.8

Table 2
Urinary corticoid-to-creatinine ratios, plasma concentrations of aldosterone (PAC) and plasma renin activity (PRA) in 11 cats with signs and symptoms suggestive of primary hyperaldosteronism. The aldosterone-to-renin ratio (ARR) was calculated from the PAC and PRA values.

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Materials and methods

Function tests

Urinary corticoid concentrations were measured by radioimmunoassay, as described previously (Rijnberk et al., 1988). The urinary corticoid concentration was related to the urinary creatinine concentration, measured by the Jaffé kinetic method (initial rate reaction, Synchron CX® Systems, Beckman Coulter Inc., Galway, Ireland) by calculating the corticoid-to-creatinine ratio (Goossens et al., 1995).

A low-dose dexamethasone suppression test (iv-LDDST) was performed with blood collection at -15 minutes, immediately before and 2, 4, 6 and 8 hours after intravenous administration of 0.01 mg dexamethasone per kg body weight (Rijnberk and Mol, 1997; Rijnberk, 1996). The test was started at 9:00 h after an overnight fast, and blood was collected for measurements of cortisol, ACTH, PAC and PRA.

Hormone measurements

Blood samples for hormone measurements were collected from the jugular vein and transferred to ice-chilled EDTA-coated tubes. Samples were centrifuged at 4°C for 10 minutes. Plasma was stored at -25°C until assayed.

Plasma ACTH was measured in an immunoradiometric assay (Nichols Institute, Wijchen, The Netherlands). The interassay coefficient of variation was 7.8%, and the sensitivity was 0.2 pmol/L. Plasma cortisol concentrations were measured by radio-immunoassay (Coat-A-Count® Cortisol, Diagnostic Product Corporation, Los Angeles, USA). The lower limit of detection was 1 nmol/L and the interassay coefficient of variation was 4-6.4%.

Aldosterone was extracted from 1 mL plasma with dichloromethane. The extracts were evaporated, redissolved in assay buffer, and aldosterone was quantitated by RIA (ICN Pharmaceuticals Inc., Costa Mesa, CA) (Boer et al., 1983). PRA was measured by incubation of 0.5 mL plasma at pH 6.0 for 1 hour at 37°C in the presence of inhibitors of angiotensinases and angiotensin I-converting enzyme. After incubation, the samples were deproteinized with acetone/ammonia 4 mol/L (9:1, v/v) and centrifuged. The super-natants were evaporated, redissolved in assay buffer, and angiotensin I was measured by RIA (using an antibody from Peninsula Laboratories Inc., Belmont, CA, and a tracer from NEN Life Sciences Products, Boston, MA) (Boer et al., 1985). The quality of assays for PAC and PRA was assessed during each run by measurement of plasma samples from large control pools. The within-assay and between-assay coefficients of variation for the PRA assay were 8 and 15%, respectively, and for the aldosterone assay 6 and 14%.

Reference values for PAC and PRA were established by taking measurements from 130 privately owned cats aged 0.3-14.5 years (median 5 years) without a history of recent (last 6 months) illness and with plasma concentrations of urea and creatinine within the reference range (Table 1). The PAC reference range was 110-540 pmol/L and that of PRA was 60-630 fmol/L/s. The ARR reference range was 0.3-3.8 (Javadi et al., 2004).

Diagnostic imaging

Ultrasonography (US) was performed with a high-definition ultrasound system (HDI 3000, Advanced Technology Laboratories, Woerden, The Netherlands). The adrenal glands were imaged through a ventral and lateral abdominal approach with the animal in supine position, using a 10-5 MHz broadband linear array transducer. Two-dimensional and M-mode echocardiography was performed through the right parasternal approach with the animal in right lateral recumbency, using a 7-4 MHz broadband phased array transducer.

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Computed tomography (CT) of the cranial abdomen was performed in the anesthetized cat with a third-generation computed tomography scanner (Tomoscan CX/S, Philips NV, Eindhoven, The Netherlands), using 120 kV, 220 mA and 4.5 s scanning time. With the animal in supine position, 5 mm thick consecutive slices were made both before and after intravenous administration of 2 mL contrast medium (Telebrix® 350, Guerbet Nederland BV, Gorinchem, The Netherlands) per kilogram of body weight. In addition, several 2 mm thick slices were made following the administration of contrast medium.

Histopathology

For histological examination, the adrenals were fixed in 10% neutral buffered formalin and routinely embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin (HE) and for neuron-specific enolase (NSE) by use of the avidin-biotin complex immunoperoxidase (ABC) method with monoclonal mouse anti-human enolase (Dako, Glostrup, Denmark). Post-mortem samples were treated in the same way, but with staining with HE and periodic acid-Schiff (PAS).

Results

Initial measurements of plasma urea and creatinine concentrations indicated mild renal insufficiency in eight of the eleven cats; in three cats, only the plasma creatinine concentration exceeded the reference range. In three other cats, both the urea and creatinine levels were within the reference range (Table 1). Six cats were hypokalemic and the other cats were normokalemic. In two of the latter cats, hypokalemia was found at subsequent blood examinations. Plasma phosphate concentrations were at the lower end of the reference range, whereas plasma calcium concentrations varied from the lower to above the upper end of the reference range (Table 1). In the six cats in which plasma magnesium concentrations were measured, the values (0.9, 0.5, 0.9, 0.8, 0.7, 0.9 mmol/L) were just within or below the reference range (0.8-1.2 mmol/L). At first presentation, none of the cats had a history or clinical findings suggestive of diabetes mellitus or hyperthyroidism. Plasma thyroxine concentrations were within the reference range (Table 1).

Hormone measurements

Urinary corticoid-to-creatinine ratios, measured in three cats on two consecutive days, were within the reference range (Table 2). At the initial investigation or shortly thereafter, plasma aldosterone concentrations exceeded the reference range in four cats. In two cats (cats 3 and 4) PRA values were in the lower end of the reference range, and in five cats (cats 1, 2, 8, 10 and 11) below the reference range. The ARRs were high in all cats (Table 2 and Figure 2).

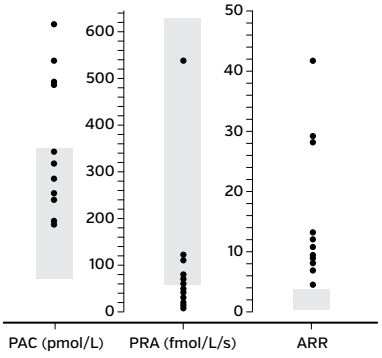

Figure 2
Concentrations of plasma aldosterone (PAC) and plasma renin activity (PRA) in 11 cats with non-tumorous (idiopathic) primary hyperaldosteronism. The aldosterone-to-renin ratio (ARR) is depicted in the right column. Shaded areas represent reference values for healthy cats.

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In the iv-LDDST, basal plasma cortisol and ACTH concentrations were within the reference range (Figure 3). In one of the cats, the basal aldosterone concentrations were higher than the reference range. The PRA concentrations were around the lower limit of the reference range. After dexamethasone administration, cortisol and ACTH concentrations declined. The cortisol values met the criterion of normocorticism (≤40 nmol/L at 8 hours after dexamethasone administration). The values of both PAC and PRA remained practically unchanged.

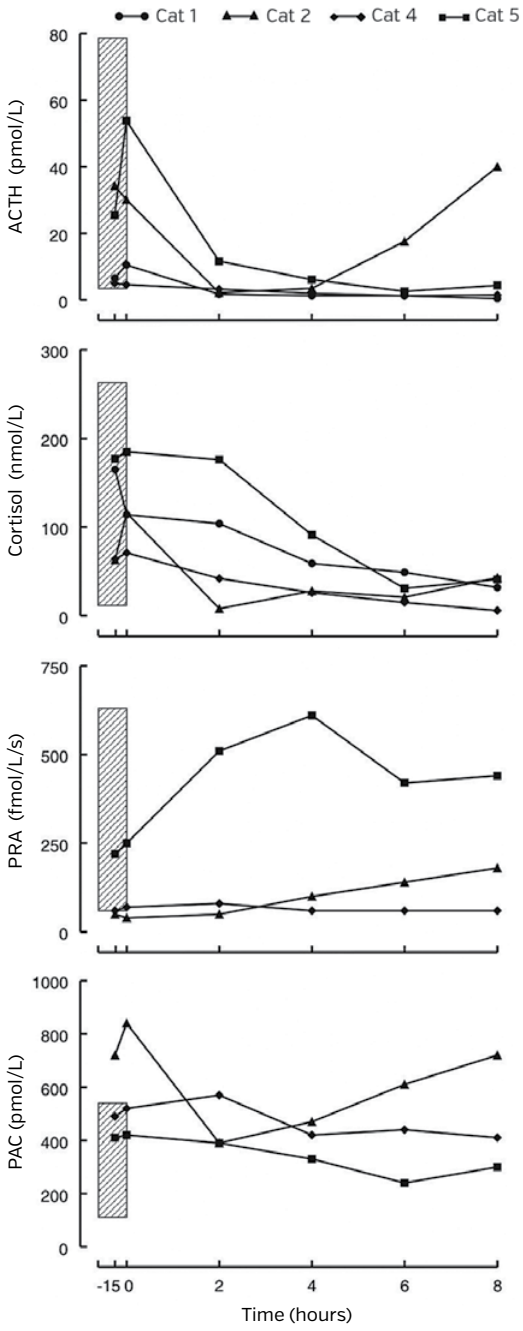

Figure 3
Plasma cortisol, ACTH and aldosterone concentrations (PAC), and plasma renin activity (PRA) in four cats with non-tumorous hyperaldosteronism before and after administration of dexamethasone (0.01 mg/kg body weight intravenously). Shaded portions represent reference values for healthy cats.

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Diagnostic imaging

In cats 1, 2, 4, 5, 6 and 7, the adrenals were visualized by ultrasonography (US), and in cats 1, 2 and 4, additionally by computed tomography (CT). In cat 1, the caudal pole of the left adrenal was somewhat thickened on both US and CT, and more echogenic than the cranial pole and than the echographic picture of the right adrenal gland. In cat 5, US revealed some calcification of the left adrenal gland. In cat 6, the cranial pole of the left adrenal was somewhat rounded and thickened on US, and there were multiple echogenic spots in both adrenals. In cat 7, the cranial poles of both adrenals were somewhat large and round on US. No abnormalities were found on US and CT in cats 2 and 4.

In cats 3 and 5, echocardiography revealed left ventricular hypertrophy with (cat 3) and without (cat 5) dilatation of the left atrium.

Clinical findings and outcome

In cat 1, the biochemical data were compatible with primary hyperaldosteronism. This together with the slight thickening of the cranial pole of the left adrenal prompted left-sided adrenalectomy. Before surgery, oral potassium supplementation was increased to 2 mmol KCl q8h. Surgery was performed via cranial midline celiotomy and was uneventful. Plasma potassium concentrations were monitored regularly for 24 hours after surgery (every 2 hours for a period of 12 hours, and thereafter every 4 hours) and were within the reference range without supplementation. At discharge, two days after surgery, the plasma potassium concentration was 3.8 mmol/L. After surgery, the plasma aldosterone concentrations decreased from 430 and 400 pmol/L to 100, 80 and 90 pmol/L at 5, 7 and 28 hours, respectively. Plasma PRA values remained low, at 20 fmol/L/s.

The cat initially did well without potassium supplementation. However, after about two months, mild hypokalemia recurred (3.0 mmol/L), probably as a result of progressive hyperplasia of the contralateral gland. Normokalemia could be maintained with resumption of the oral potassium supplementation (1 mmol KCl q12h). Although the owner thought the cat was no longer blind, ophthalmic examination revealed that pupils were dilated and pupillary light responses were absent. The neuroretina of the left eye was still detached and separated from the ora ciliaris retinae at the periphery. In the right eye, the retina had reattached but showed signs of severe degeneration, as evidenced by retinal vascular attenuation and generalized tapetal hyperreflectivity. The cat did reasonably well until 1.75 years after presentation, when we were informed that the cat had been euthanized elsewhere after sudden-onset paralysis of the hind limbs.

As in the first case, in cat 2, ophthalmologic crisis and high systolic blood pressure were the reasons for investigation of adrenocortical function. Once the diagnosis of primary hyperaldosteronism had been firmly established and hypokalemia had developed (Table 1), treatment with the aldosterone antagonist spironolactone (Aldactone®, Searle Nederland BV, Maarssen, The Netherlands) was started at a dose of 6.25 mg q12h PO. Initially, this normalized the plasma potassium concentration, but the dose had to be doubled a few months later to maintain normokalemia. According to the owner, the cat did well on this treatment in that the appetite and interest in the environment improved; however, the gradually increasing plasma concentrations of urea and creatinine (Figure 1) and the associated malaise made the owner decide for euthanasia.

In cat 3, there was not only blindness due to bilateral retinal detachment with hemorrhage, but also left ventricular hypertrophy (with left atrial dilatation) and lung edema. Treatment with a beta-adrenergic blocker was started. Two days later, the cat died. In cat 4 with potassium suppletion, no further attacks of flaccid paresis occurred.

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The cat did well until about 9 months after referral, when a uremic crisis occurred (Figure 1). The owner requested euthanasia.

Both cats 5 and 6 are currently being treated with spironolactone and are doing reasonably well. In cat 5, ultrasonographic examination had also revealed left ventricular hypertrophy, and consequently beta-adrenergic blocker therapy was added to the medication regimen. Plasma concentrations of urea and creatinine gradually increased without a concurrent rise in plasma phosphate concentrations. This was also true for cat 7, in which, at the owner's request, treatment was confined to potassium supplementation. In cats 8-11, medication has only recently been started.

Pathology

Macroscopically, the adrenal glands (cat 1, surgical specimen; cats 2 and 4, post-mortem material) had no abnormalities. On histological examination, the cortex was composed of multiple small hyperplastic nodules consisting of large, pale, vacuolated cells. These nodules stained diffusely and markedly positive for NSE. In healthy control cats, NSE staining was confined to the narrow zona glomerulosa with some vague staining of the outer parts of the zona fasciculata. There was no staining of the zona reticularis and the inner parts of the zona fasciculata (Figure 4).

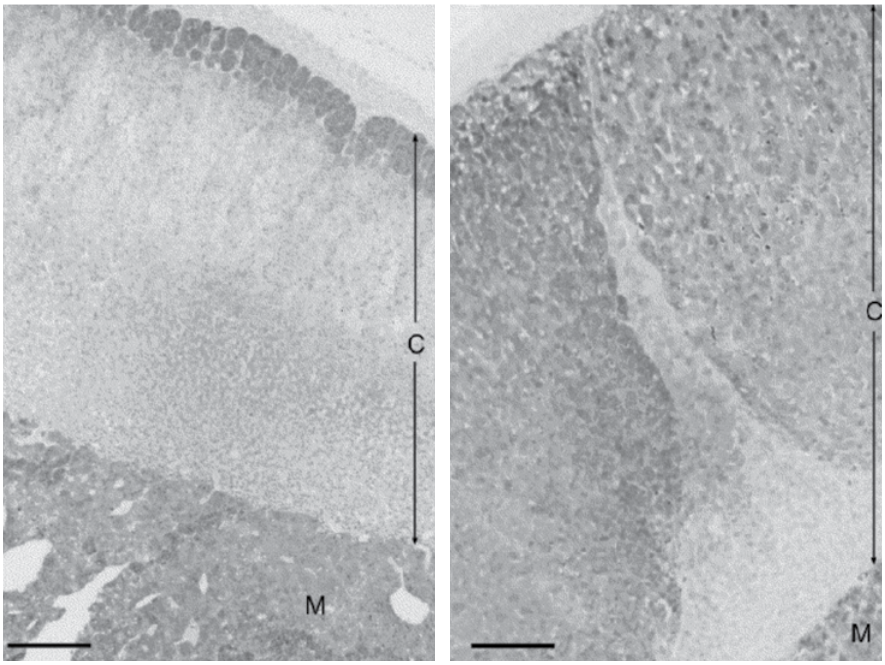

Figure 4
Two adrenal glands stained with neuron-specific enolase (NSE). In the healthy control cat (left), the staining of the cortex (C) is confined to the zona glomerulosa with some vague staining of the outer parts of the zona fasciculata. In cat 2 with primary hyperaldosteronism (right), the cortex mainly consists of multiple hyperplastic nodules, staining positively for NSE. In both sections, there is similar staining of the adrenal medulla (M). Bar = 200 µm.

At post-mortem examination, the kidneys of cats 2 and 4 were small and grayish with a finely granular surface and an increased consistency. Histologically, there were coalescing areas of moderate interstitial fibrosis, particularly in the deeper cortex and the cortico-medullary junction, with slight lymphocytic infiltration. These fibrotic areas were associated with sclerotic atrophic glomeruli and glomeruli with hyaline fibrosis of Bowman's capsule, as well as slight segmental membranoproliferative glomerulonephritis, atrophic tubules with thickened basement membranes, and incidental proteinaceous casts in the tubules (Figure 5). The radiate arteries were clearly abnormal compared with those of healthy cats.

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In the control kidneys, the arteries were straight and well-delineated, with thin walls (Figure 6). The arteries in the kidneys of the cats with primary hyperaldosteronism had a coiling pattern with multiple cross- and longitudinal sections, thick walls with cellular proliferation and formation of onion-like configurations and some hyaline deposits (Figure 7).

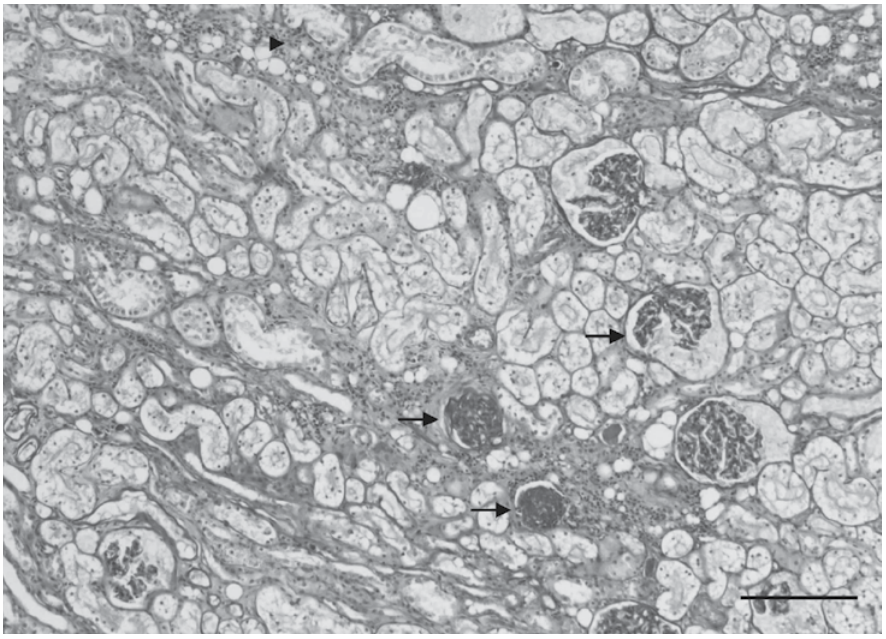

Figure 5
Kidney of cat 4 with primary hyperaldosteronism. In this section of the deeper cortex and corticomedullary junction, fibrotic areas with sclerotic atrophic glomeruli (arrows) and a slight mononuclear inflammatory infiltrate (arrow heads) are visible. Periodic acid-Schiff (PAS), bar = 200 µm.

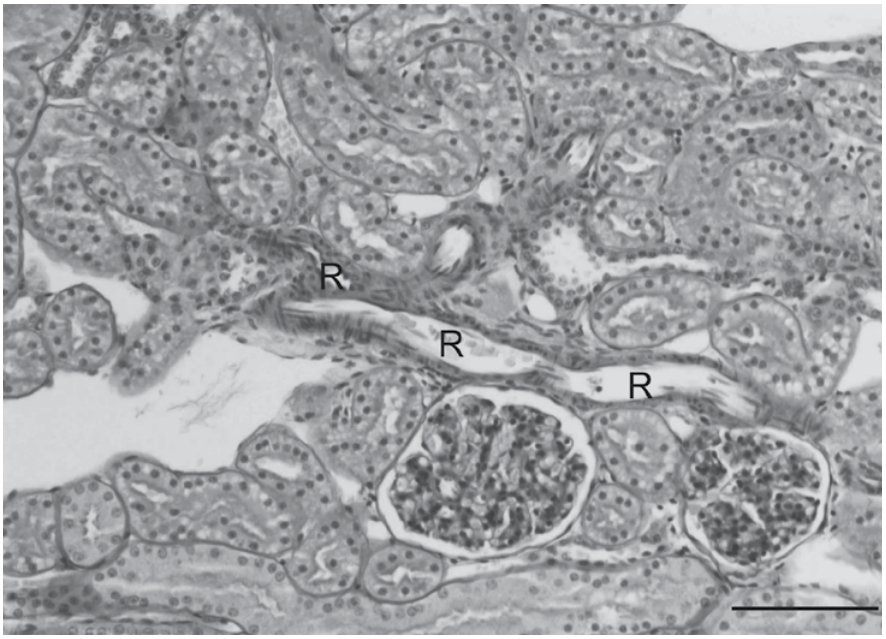

Figure 6
Kidney of a healthy control cat depicting a radiate artery (R) with a straight course and a thin, well-delineated muscular wall. PAS, bar = 100 µm.

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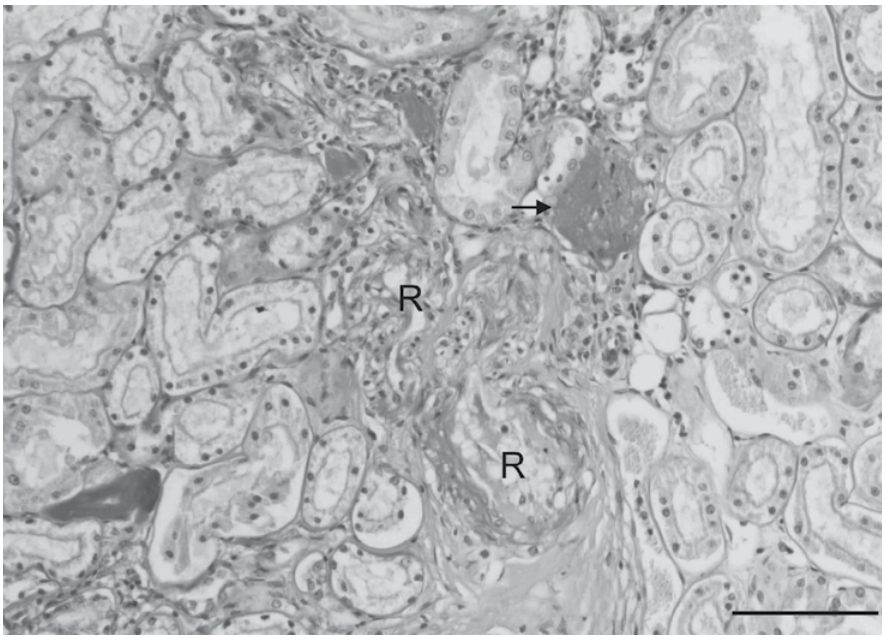

Figure 7
Section of a kidney from cat 2 with primary hyperaldosteronism showing a coiled radiate artery in multiple cross-sections (R) with an increased wall thickness, formation of onion-like configurations, and hyaline PAS-positive deposits (arrow). PAS, bar = 100 µm.

Discussion

In principle, the diagnosis of hyperaldosteronism should be based on plasma aldosterone concentrations exceeding the reference range. The upper limit of PACs found in the 130 privately owned cats (540 pmol/L) was comparable to that reported earlier for cats kept in a research center (700 pmol/L, n=148) (Yu and Morris, 1998) and for household cats (430 pmol/L, n=14) (Mackay et al., 1999). In privately owned cats, there is little variation in salt intake, an important determinant for aldosterone secretion (Papanek et al., 1993). Cats are mostly fed manufactured foods, for which the recommendations of the National Research Council (NRC, 1986), USA (0.5 g Na/kg diet), may be followed (NRC, 1986). However, recently it has been pointed out that cats have a higher sodium requirement than this recommendation (Yu and Morris, 1999). In both the healthy household cats and the clinical cases, this was certainly met, because now manufactured foods guarantee a relatively constant sodium and potassium content of 0.2-0.4% and 0.6-0.7%, respectively on an as is basis, as recommended by the Association of American Feed Control Official (AAFCO, 1994). The reference values used in this study are very similar to the reference values established in the same laboratory for humans on free salt intake: PRA 100-650 fmol/L/s; PAC 80-450 pmol/L; aldosterone-to-renin ratio 0.4-3.2.

Four of the eleven cats investigated had an elevated PAC, although concentrations were relatively low when compared to those of recently reported cases of aldosteronoma in cats (Flood et al., 1999; MacKay et al., 1999; Moore et al., 2000; Rijnberk et al., 2001). However, PAC values have to be interpreted against the background of another important determinant of aldosterone secretion, that is plasma potassium concentration. K^+-ions directly stimulate the zona glomerulosa cells to secrete aldosterone, probably by activating both voltage-dependent calcium channels and locally produced angiotensin II (Vassilev et al., 1992; Kifor et al., 1991). As hypokalemia is a predominant factor in lowering plasma aldosterone concentration (Kolloch et al., 1996), the aldosterone values in the present clinical cases should be regarded as inappropriately high.

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In addition, the PRA values must be taken into consideration. The combination, such as in the present cats, of high-normal or elevated aldosterone levels and low PRA levels indicates persistent aldosterone synthesis in the presence of minimal or absent stimulation from the renin-angiotensin system. These changes are characteristic of primary hyperaldosteronism, first described in humans in 1955 by Conn (Conn, 1955). Hiramatsu et al. first suggested using the aldosterone/PRA ratio as an aid in identifying humans with primary hyperaldosteronism (Hiramatsu et al., 1981). This ratio is now commonly used as a screening procedure in humans and has also proven to be useful in diagnosing primary hyperaldosteronism in patients with normokalemia and/or aldosterone levels in the upper end of the reference range (Lim et al., 1999; Gordon et al., 1993). In the present cats, the ARRs exceeded the reference range on one or more occasions. The fact that the ratios were not persistently elevated underlines the importance of repeated measurements of both PAC and PRA (Gill, 2001).

Once the diagnosis of primary hyperaldosteronism has been established, further characterization is needed to enable adequate treatment. Several sub-types of primary hyperaldosteronism have been identified in humans, the most common being aldosterone-producing tumors and bilateral hyperplasia of the zona glomerulosa (Young, 1997). In addition, there are forms of familial hyperaldosteronism. One of these inherited forms, glucocorticoid-remediable hyperaldosteronism, is caused by an unequal crossover between the gene for aldosterone synthase and the gene for 11β-hydroxylase. This results in a chimeric gene that has aldosterone synthase activity but is regulated by ACTH rather than angiotensin II (Lifton et al., 1992; Pascoe et al., 1992). In our cats, a low dose of dexamethasone caused the plasma ACTH concentrations to decrease, whereas PAC did not decrease. This, together with the sporadic occurrence at a relatively old age rather than familial occurrence at a young age makes glucocorticoid-remediable hyperaldosteronism very unlikely.

Our cats most likely had sporadic primary hyperaldosteronism due to bilateral hyperplasia of the zona glomerulosa. This was confirmed in the three cats in which the adrenals were examined histologically. All adrenal cortices had micronodular hyperplasia of the zona glomerulosa, similar to that seen in humans with hyperaldosteronism due to bilateral hyperplasia (Gordon et al., 1991). The etiology of this (idiopathic) hyperaldosteronism has not been established; a circulating stimulatory factor is thought to be responsible for hyperfunction of the zona glomerulosa. The factor has not been identified. A peptide of pituitary origin, possibly a fragment of pro-opiomelanocortin (POMC), has been implicated by some authors but not by others (Griffing et al., 1985; Miyamori et al., 1990).

Another explanation for the development of micronodular hyperplasia of the zona glomerulosa could be the involvement of the pituitary-gonadal axis. In postmenopausal woman, estrogen-replacement therapy decreases heart rate and blood pressure (Harvey et al., 1999). Results of experiments in ovariectomized rats indicate that estradiol decreases adrenal expression of angiotensin-II receptors, leading to attenuated aldosterone responses to stimulation by angiotensin II. The mechanism underlying this (beneficial) effect is not clear. It has been suggested that estradiol directly regulates adrenal angiotensin-II receptor transcription and/or indirectly modulates this transcription by modifying the expression of a newly discovered cytosolic protein (Roesch et al., 2000).

It may also be speculated that the effects of estrogens on mineralocorticoid production are exerted via feedback at pituitary level. In this respect, a comparison with

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hyperadrenocorticism in ferrets urges itself. In this species, castration leads to a high incidence of LH-dependent bilateral adrenocortical hyperplasia and tumor. Apparently with time, the persistently high circulating gonadotropin levels, due to the absence of feedback by gonadal steroids, lead to increased expression of LH receptors in the adrenal cortex (Schoemaker et al., 2002). It is tempting to speculate that in (idiopathic) feline hyperaldosteronism also, LH plays a role. In fact, we have recently found that the ARR is significantly higher in castrated cats than in intact cats (Javadi et al., 2004).

In the six cats that could be followed for some time, there were gradual increases of the plasma values of urea and creatinine, indicating progression of renal insufficiency. This progression is not just a matter of aging, as in a recent study in healthy adult cats, plasma creatinine and urea concentrations did not change with age (Javadi et al., 2004). Remarkably, in the present cats, the progression of renal insufficiency was not associated with a concomitant rise in the plasma phosphate concentrations, as is usual in advanced renal failure. In fact, there was a tendency to hypophosphatemia. This has also been observed in humans with hyperaldosteronism and can be considered as an escape from chronic mineralocorticoid-induced sodium retention. The volume expansion-induced resetting of the proximal glomerulotubular balance leads to an increased fractional clearance of calcium and phosphate (Boer et al., 1987). In turn, the negative calcium balance and particularly the associated tendency to hypocalcemia may give rise to hypersecretion of parathyroid hormone. The phosphaturic effect of hyperparathyroidism will contribute to the low plasma phosphate concentrations.

However likely this explanation may seem, the hypercalcemia observed in some of the cats is suggestive of an associated occurrence of hyperaldosteronism and hyperparathyroidism, as has been reported in humans (Ferriss et al., 1983). Investigations in ten hypertensive patients with primary hyperaldosteronism made the authors conclude that parathyroid hypersecretion is a common feature of primary hyperaldosteronism, and that there may be a relationship between the activity of the renin-aldosterone system and parathyroid pathophysiology (Resnick and Laragh, 1985). The latter suggestion has been substantiated by the discovery of a parathyroid hypertensive factor in low-renin forms of hypertension (Resnick et al., 1993; Lewanczuk et al., 1994). The possible role of this factor in cats with hyperaldosteronism needs to be studied.

The histopathologic changes of the kidneys were identical to those reported for humans with hyperaldosteronism, i.e. hyaline arteriolar sclerosis, glomerular sclerosis, tubular atrophy and interstitial fibrosis (O'Neal et al., 1970; Danforth et al., 1977; Grady et al., 1996). At the time of necropsy, the two cats (cats 2 and 4) had clear-cut renal insufficiency. However, when primary hyperaldosteronism was diagnosed, the urea and creatinine concentrations in plasma were still within the respective reference ranges. Moreover, some of the other cats were known to have low plasma potassium concentrations (e.g. cats 7 and 8) before PAC and PRA were measured, which make it likely that it was rather the hyperaldosteronism that caused kidney disease than the reverse.

In previously described cats with hyperaldosteronism due to adrenal tumors, the situation was much less dominated by progressive renal insufficiency, even though plasma concentrations of aldosterone were much higher in the former than in the latter cats with non-tumorous hyperaldosteronism. This may be due to the different PRA levels. In the present study, in most instances, PRA was not fully suppressed, whereas in the cases with aldosterone-producing tumors, the extremely high aldosterone levels caused complete PRA suppression. As early as the 1970s, it was concluded that

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a low-renin status protects against vascular complications (Laragh, 1973; Laragh et al., 1975), probably as a result of the associated low angiotensin II concentrations. More recently, the significance of PRA escape from suppression by hyperaldosteronism has been re-emphasized. In humans, severe arterial hypertension caused by primary hyperaldosteronism may lead to arteriosclerotic kidney damage that counteracts renin suppression and accelerates the progression of vascular changes (Oelkers et al., 2000). When this concept is applied to our cats, one may argue that the mild (idiopathic) hyperaldosteronism initially, and then for a relatively long time, did not lead to complete PRA suppression, thereby allowing both aldosterone and angiotensin II to affect the renal tissue. Once established, renovascular damage may elicit renin release even in the presence of gradually increasing aldosterone concentrations. Thus, in relatively mild hyperaldosteronism, the kidneys are exposed persistently to two important mediators of vascular changes and fibroproliferative destruction.

The cats were initially treated symptomatically, with potassium supplementation. This prevented attacks of muscular weakness and restored normokalemia. Later, in recognition of the relevance of primary hyperaldosteronism, the aldosterone antagonist spironolactone was introduced. The results of these treatments cannot be assessed as there was no strict protocol. Similar to what is foreseen in man, in cats randomized studies should be initiated to delineate the potential renal-protective effect of a specific aldosterone receptor antagonist (Epstein, 2001). In addition, there is a need for systematic studies of ARRs in cats with renal disease, with and without abnormalities in electrolytes. These studies may provide further insight into the possible pathogenetic role of the renin-angiotensin-aldosterone system in the progression of renal disease.

In conclusion, the non-tumorous form of primary hyperaldosteronism in cats is very similar to “idiopathic” primary hyperaldosteronism in humans. The condition is associated with progressive renal disease, which may in part be due to the often incompletely suppressed plasma renin activity.

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04

Plasma aldosterone-to-renin ratio in cats with chronic kidney disease

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Abstract

Background: Aldosterone is thought to be a mediator of progression of chronic kidney disease (CKD) in humans, and progression of CKD has been observed in cats with primary hyperaldosteronism.

Objectives: To determine the proportion of cats with an elevated plasma aldosterone-to-renin ratio (ARR) in a group of cats with CKD.

Animals: Fifty-one privately owned cats with a plasma creatinine concentration >140 µmol/L on two or more occasions were enrolled.

Methods: In this prospective clinical study the ARR was determined.

Results: ARR was elevated in seven cats (14%). The suspicion of primary hyperaldosteronism was further supported by an increased plasma aldosterone concentration (PAC) in three of these cats. Based on the ARR and PAC, secondary hyperaldosteronism was suspected in five other cats.

Conclusions and clinical importance: Inappropriately high aldosterone secretion is apparently not uncommon in cats with CKD. Its detection provides options for treatment which may be able to slow down the rate of loss of kidney function.

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Introduction

Primary hyperaldosteronism, also termed primary aldosteronism, low-renin hyperaldosteronism, and Conn's syndrome, is an adrenocortical disorder characterized by autonomous aldosterone secretion by either neoplastic or hyperplastic zona glomerulosa tissue (Galac et al., 2010). Primary hyperaldosteronism was first described in a cat in 1983 (Eger et al., 1983), and was initially regarded a rare disease in cats. However, the number of case reports has risen considerably in the past 15 years (Flood et al., 1999; MacKay et al., 1999; Moore et al., 2000; Bruyette, 2001; Rijnberk et al, 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005; Reimer et al., 2005; Rose et al., 2007; Djajadiningrat-Laanen et al., 2008; Briscoe et al., 2009; Renschler and Dean, 2009; Smith et al., 2012; Willi et al., 2012), and increased awareness of the disease will probably lead to a further increase in detected cases.

The ratio between the plasma aldosterone concentration (PAC) and the plasma renin activity (PRA), also referred to as the aldosterone-to-renin ratio (ARR), is used as a screening test for primary hyperaldosteronism in cats (Javadi et al., 2004; Javadi et al., 2005; Briscoe et al., 2009; Willi et al., 2012). Using the ARR is more appropriate than relying on an increased PAC alone, since PAC can also be elevated as a physiological response to increased renin levels. In case of secondary hyperaldosteronism increased aldosterone secretion is a result of increased renin secretion, resulting in an ARR that is usually within the reference range. Moreover, cats with primary hyperaldosteronism have been reported in which PAC was within the reference range (Javadi et al., 2005), but suppressed PRA resulted in an elevated ARR. These observations strongly support that the ARR is a better positive screening test for primary hyperaldosteronism in cats than PAC alone, and argue against the requirement of an elevated PAC in addition to an elevated ARR for a positive screening test for primary hyperaldosteronism.

Inappropriately high aldosterone secretion has some serious implications, the most important being related to actions of aldosterone on the kidneys. Excessive mineralocorticoid receptor activation in the epithelial cells of the distal nephron results in potassium depletion and sodium and water retention, thereby leading to hypokalemia or systemic arterial hypertension, or both. Hence cats with primary hyperaldosteronism are typically presented with muscle weakness due to hypokalemic myopathy and/or complications of arterial hypertension, such as acute blindness associated with retinal detachment and/or intraocular hemorrhage (for an overview, see Djajadiningrat-Laanen et al., 2011).

In addition to these overt and often alarming clinical signs of mineralocorticoid excess, primary hyperaldosteronism may also lead to tissue damage of a more insidious nature, e.g. as a consequence of aldosterone-induced vasculopathy. Aldosterone has proinflammatory and profibrotic properties, and thrombotic and vascular proliferative lesions in the heart and kidneys have been demonstrated in response to exogenous aldosterone administration in rodent models (Rocha et al., 1999; Sun et al., 2002). There is increasing evidence that these non-epithelial actions of aldosterone can promote and accelerate progressive kidney disease in humans (Farquharson and Struthers, 2002; Hollenberg, 2004). Progressive CKD has also been reported in cats with primary hyperaldosteronism (Javadi et al., 2005). Primary hyperaldosteronism may cause kidney damage through two mechanisms: (1) arterial hypertension, and (2) aldosterone-induced vasculopathy.

Chronic kidney disease (CKD), defined as the presence of functional or structural abnormalities in one or both kidneys for three months or more (Polzin, 2010), is a

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common finding in feline patients. It primarily affects middle-aged to elderly cats (Elliott and Barber, 1998), and especially in its more advanced stages has a major impact on both the quality of life and life expectancy (Syme et al., 2006; King et al., 2007; Boyd et al., 2008). In many cases the primary cause of renal dysfunction is unknown and therapy is at best symptomatic, renal diets being the best evidence-based option for reducing the number of uremic episodes and the risk of kidney-related mortality (Roudebush et al., 2009). Finding and treating the primary cause of CKD should be more effective in delaying the loss of kidney function, but is not often pursued or possible.

In this paper we report on the prevalence of inappropriate aldosterone secretion in a group of cats with CKD, using the plasma aldosterone-to-renin ratio as a case-finding test.

Materials and methods

Patients

Fifty-one client-owned cats which had a plasma creatinine concentration above 140 µmol/L on two or more occasions were enrolled in the study, with the informed consent of their owners. Cats with elevated plasma thyroxine concentrations and cats with diabetes mellitus were excluded. Since elevated plasma creatinine concentrations have been reported in Birman cats without any clinical signs of kidney disease (Gunn-Moore et al., 2002), Birman cats with an elevated plasma creatinine concentration but no other clinical signs or biochemical abnormalities compatible with chronic kidney disease were excluded as well. Forty-six cats were recruited from 18 private veterinary practices in The Netherlands and five cats had been referred for CKD to the Utrecht University Clinic for Companion Animals. Their age, breed and gender are given in Table 1.

		sPHA (n=7)	sSHA (n=5)	NHA (n=39)	Total (n=51)
Age (years)		15 (10-18)	7 (6-13)	13 (7-19)	13 (6-19)
Breed	Domestic shorthair	6	3	26	35
	Birman			4	4
	Somali	1		1	2
	British shorthair		1	1	2
	Persian			2	2
	Norwegian forest cat			1	1
	Siamese			1	1
	Cross-bred		1	3	4
Gender	Neutered male	3	2	24	29
	Neutered female	4	3	15	22

Table 1
Age (median and range), breed and gender of 51 cats with chronic kidney disease and a plasma aldosterone concentration and an aldosterone-to-renin ratio suggesting primary hyperaldosteronism (sPHA), secondary hyperaldosteronism (sSHA), or no hyperaldosteronism (NHA).

Thirty cats were at International Renal Interest Society (IRIS) CKD stage 2 (plasma creatinine concentration 140-249 µmol/L), 17 at IRIS CKD stage 3 (plasma creatinine concentration 250-439 µmol/L), and 4 at IRIS CKD stage 4 (plasma creatinine concentration >440 µmol/L) (Polzin, 2010). Forty-two of the cats were on a diet (40 on a renal diet and two on a struvite urolithiasis prevention diet) and 21 received oral medication: benazepril (n=11), ipakitine (n=5), amlodipine (n=4), methimazole (n=2), ranitidine (n=2), atenolol (n=1), cisapride (n=1), clomipramine (n=1), lactulose (n=1), l-thyroxine (n=1), metoclopramide (n=1), mirtazapine (n=1), pancreatic enzymes (n=1)

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and potassium gluconate (n=1). The diet and medication were continued as prescribed, with the exception of the angiotensin-converting enzyme inhibitor benazepril, which was withheld for at least seven days prior to the examination.

The examination included measurement of arterial blood pressure, slit lamp and ophthalmoscopic examination, and blood and urine examinations. The owners were instructed to withhold food for at least 8 hours before the examination.

Systolic blood pressure measurements and ophthalmic examination

Arterial blood pressure was measured before any other examination, using a Doppler flow detector (Parks Model 811-B ultrasonic Doppler flow detector, Parks Medical Electronics Inc., Aloha, OR, USA) with a 5 cm-wide cuff (Babyphon® infant, Rudolf Riester GmbH & Co.KG, Jungingen, Germany) and a handheld sphygmomanometer (Precisa® N, Rudolf Riester GmbH & Co.KG, Jungingen, Germany). The measurement was performed following a protocol that included acclimatization for 10 minutes and minimal restraint of the cat, standing or lying in sternal recumbency (Brown et al., 2007). The mean of a minimum of three consecutive measurements with less than 20% variation was recorded. Cats with systolic pressures of 160-180 mmHg were examined for ophthalmic signs of arterial hypertension. Slit lamp and ophthalmoscopic examination were performed in a darkened room with a direct ophthalmoscope (3.5 V Coaxial Ophthalmoscope, Welch Allyn, Delft, The Netherlands). Systemic arterial hypertension was defined as mean systolic pressure >180 mmHg, or >160 mmHg in the presence of compatible ocular signs such as hemorrhages (anterior chamber, vitreous, retina or subretinal space), retinal vascular tortuosity, and multifocal to complete retinal detachment.

Blood collection and laboratory examination

Blood (8 mL) was obtained by jugular venipuncture. Three mL were collected in a heparin-coated tube for measurement of plasma concentrations of urea, creatinine, sodium, potassium, calcium, phosphate, albumin, total thyroxine and fructosamine. Plasma total thyroxine concentration was measured to rule out hyperthyroidism (reference range: 15-45 nmol/L), and the plasma fructosamine concentration to rule out diabetes mellitus in cats in which urinary glucose could not be measured (reference range: 156-260 µmol/L). One mL blood was collected in an EDTA-coated tube for measurement of hematocrit and reticulocytes. All measurements were performed at a single laboratory.

For measurement of plasma aldosterone concentration (PAC) and plasma renin activity (PRA), 4 mL blood collected in an ice-chilled EDTA-coated tube was stored on ice until centrifuged. The plasma was removed immediately and stored at -20°C. PAC and PRA were measured at a single laboratory as described previously (Boer et al., 1983), and validated for the cat (Javadi et al., 2004). The plasma aldosterone-to-renin ratio (ARR) was the quotient of the PAC in pmol/L divided by the PRA in fmol/L/s. PRA values below the detection limit were set at 40 fmol/L/s in order to allow calculation of the minimum ARR. The reference ranges for PAC (110-540 pmol/L), PRA (60-630 fmol/L/s) and ARR (0.3-3.8) have been reported previously (Javadi et al., 2004). Based on the ARR and PAC, cats were classified as: (1) no hyperaldosteronism (NHA) (neither PAC nor ARR elevated); (2) suspected primary hyperaldosteronism (sPHA) (ARR elevated); or (3) suspected secondary hyperaldosteronism (sSHA) (PAC elevated, ARR within reference range).

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Urine collection and laboratory examination

Urine was collected by the owner from the cat's own litter box, which had been cleaned, dried and bedded with a non-absorbent cat litter (Katkor®, Reinvet Products, Utrecht, The Netherlands). The sample was collected within 24 hours prior to the examination and was refrigerated until processed. If the owner was unable to collect urine, it was obtained by cystocentesis. A 3 mL sample was used to measure urine specific gravity (SG), pH, hemoglobin, glucose, sediment, and the concentrations of creatinine and total protein.

Statistics

Statistical analyses were performed using IBM® SPSS® Statistics version 21.0.0 (IBM® Corporation, Armonk, NY, USA). The Kolmogorov-Smirnov test and the Shapiro-Wilk test were used to test the data for normal distribution. Two groups were compared using Levene's test for equality of variances and a two-tailed independent-samples t-test for equality of means for normally distributed data, and a two-tailed Mann-Whitney U Test for data not normally distributed. Three groups were compared using one-way between-groups ANOVA with post-hoc tests for normally distributed data, and a Kruskal-Wallis test for data not normally distributed. Systolic blood pressure was analyzed only in cats that had received no antihypertensive medication, plasma potassium concentration was analyzed only in cats that had received no potassium supplements, and the urinary protein-to-creatinine ratio was analyzed only in cats that had no abnormalities in the urinary sediment. The Spearman correlation coefficient was used to analyze data not normally distributed. $P<0.05$ was considered significant. Data are presented as median and range.

Results

Twelve of 51 cats had abnormalities in PAC, PRA and/or ARR indicative of hyperaldosteronism (sPHA and sSHA, Table 2). Seven of these cats were suspected of having primary hyperaldosteronism (sPHA), and five of having secondary hyperaldosteronism (sSHA). The remaining 39 cats had no evidence of hyperaldosteronism (NHA) (Table 1).

In three sPHA cats with an elevated ARR, PAC was also elevated. In five sPHA cats PRA was below the limit of detection. PAC and PRA measurements were repeated in two of these five cats. The first and second results in the first cat were: PRA <40 and 52 fmol/L/s, PAC 890 and 850 pmol/L, and ARR 22.2 and 16.3. In the second cat they were: PRA <40 and 230 fmol/L/s, PAC 220 and 410 pmol/L, and ARR 5.5 and 1.8.

Cats suspected of having primary hyperaldosteronism (sPHA) had a significantly higher ARR (5.5, $P<0.01$) and PAC (330 pmol/L, $P=0.040$), and a significantly lower PRA (40 fmol/L/s, $P<0.01$) and plasma urea concentration (10.5 mmol/L, $P=0.016$) than those not having primary hyperaldosteronism (NHA and sSHA combined: ARR=0.8, PAC=175 pmol/L, PRA=200 fmol/L/s, plasma urea concentration=16.5 mmol/L). Differences between these two groups in other parameters were not significant.

In comparison with NHA and sPHA cats, those suspected of having secondary hyperaldosteronism (sSHA) were younger ($P=0.013$) (Table 1), and had a significantly higher plasma urea concentration ($P=0.014$), plasma creatinine concentration ($P=0.014$), PAC ($P<0.01$) and PRA ($P<0.01$), and a significantly lower urine SG ($P=0.043$) (Table 2). The difference in ARR between them was significant ($P<0.01$), but differences in other parameters were not. PAC, PRA and ARR were not significantly correlated with any other parameter.

Hypokalemia was found in 10 NHA cats, 2 sPHA cats (despite oral potassium supplementation in one) and 2 sSHA cats. Neither differences in the proportion of cats

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Parameter (units)	sPHA	sSHA	NHA	Reference range
PAC (pmol/L)	330 (170-890) [7]	1040 (660-2300) [5]	140 (29-450) [39]	110-540
PRA (fmol/L/s)	40 (40-100) [7]	370 (200-640) [5]	200 (55-780) [39]	60-630
ARR	5.5 (4.1-22.2) [7]	3.3 (1.9-3.7) [5]	0.7 (0.1-3.3) [39]	0.3-3.8
Creatinine (µmol/L)	211 (169-249) [7]	395 (252-716) [5]	223 (168-524) [39]	76-164
Urea (mmol/L)	10.45 (9.0-14.3) [6]	21.1 (15.2-46.0) [5]	16.45 (7.1-32.2) [28]	6.1-12.8
Sodium (mmol/L)	151 (149-154) [7]	150 (146-155) [5]	150 (144-155) [38]	146-158
Potassium (mmol/L)	3.6 (3.1-3.8) [6]	3.5 (3.0-4.2) [5]	3.7 (2.7-5.3) [36]	3.4-5.2
Calcium (mmol/L)	2.66 (2.48-2.85) [7]	2.70 (2.57-3.26) [5]	2.62 (2.21-3.31) [39]	2.36-2.86
Phosphate (mmol/L)	1.27 (0.89-1.61) [7]	1.87 (1.16-3.82) [5]	1.28 (0.87-2.62) [39]	0.89-2.05
Albumin (g/L)	26 (23-27) [6]	26.5 (24-29) [4]	26 (20-32) [25]	25-34
Hematocrit (L/L)	0.32 (0.23-0.38) [6]	0.27 (0.22-0.32) [5]	0.30 (0.19-0.42) [35]	0.28-0.47
Reticulocyte count (%)	0.05 (0.0-0.2) [6]	0.1 (0.0-0.2) [5]	0.1 (0.0-0.4) [33]	<1.3
Urine SG	1.030 (1.018-1.045) [5]	1.013 (1.012-1.031) [5]	1.019 (1.011-1.050) [37]	>1.020
UPC	[0]	0.20 (0.19-0.64) [3]	0.23 (0.07-0.56) [8]	<0.4

Table 2
Blood and urine findings in 51 cats with chronic kidney disease and a plasma aldosterone concentration and an aldosterone-to-renin ratio suggestive of primary hyperaldosteronism (sPHA), secondary hyperaldosteronism (sSHA) or no hyperaldosteronism (NHA). The values are: median (range) [n].

with hypokalemia, nor differences in the plasma potassium concentration among the three groups were significant.

Based on arterial blood pressure and ophthalmic findings, systemic arterial hypertension was diagnosed in 24 cats: 18 NHA cats (despite antihypertensive medication in three), 4 sPHA cats (despite antihypertensive medication in one) and 2 sSHA cats. In NHA cats not receiving antihypertensive medication the median systolic blood pressure was 164 mmHg (range 124-238 mmHg, n=31), in sPHA cats it was 180 mmHg (range 131-240 mmHg, n=6), and in sSHA cats it was 180 mmHg (range 170-245 mmHg, n=5). Neither differences in the proportion of cats with arterial hypertension nor differences in systolic blood pressure among the three groups were significant.

Discussion

In this study, 51 privately owned cats with CKD were screened for primary hyperaldosteronism by measuring the ARR. A remarkably high 14% had an elevated ARR, suggesting that primary hyperaldosteronism is more common in cats with CKD than has previously been assumed. An additional 10% with an ARR within the reference

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range had an elevated PAC, suggestive of secondary hyperaldosteronism. The finding of abnormalities in the ARR and/or PAC in nearly a quarter of the examined cats suggests that aldosterone and renin measurements should be included in the diagnostic evaluation of cats with CKD.

There is at present no 'gold standard' test with uniform reference values and known sensitivity and specificity for evaluation of the renin-angiotensin-aldosterone system (RAAS) in cats. This complicates the interpretation of abnormalities in the RAAS in cats with CKD, but the ARR may be helpful in the diagnosis of primary hyperaldosteronism. Review of the 23 documented cases of feline primary hyperaldosteronism in which both PAC and PRA were determined suggests that the ARR has a high sensitivity for detecting primary hyperaldosteronism. It was above the reference range in 7 of the cats (Javadi et al., 2005), and in the remaining 16 cats, for which a reference range for the ARR was not specified, PAC was elevated and PRA was below or within the reference interval (Eger et al., 1983; Flood et al., 1999; Moore et al., 2000; Bruyette, 2001; Rijnberk et al., 2001; Briscoe et al., 2009; Smith et al., 2012). In one cat the ARR was within the reference range when first measured but was found to be elevated upon repeat measurement (Javadi et al., 2005). Histopathological examination revealed that this cat had bilateral micronodular hyperplasia of the zona glomerulosa. This implies that in a cat with clinical signs of primary hyperaldosteronism finding a normal ARR does not exclude primary hyperaldosteronism, and measurements of PAC and PRA may have to be repeated. It also raises the possibility that some CKD cats with primary hyperaldosteronism have been missed in the present study.

A second reason for a potentially underestimated prevalence of primary hyperaldosteronism in this group of CKD cats is a medication effect. For this study, medications with a direct effect on the feline RAAS were discontinued. Although no such effect has been documented for amlodipine and atenolol in cats, these antihypertensive medications have been reported to potentially influence ARR results in humans: atenolol may falsely increase the ARR and amlodipine may falsely decrease it (Mulatero et al., 2002). In the four cats receiving atenolol and/or amlodipine, the medications were considered to be indispensable for control of arterial blood pressure and prevention of further hypertension-induced damage to the kidneys, eyes, heart and brain, and were therefore intentionally continued. One cat receiving oral amlodipine had an elevated ARR based on an elevated PAC and pronounced suppression of the PRA, and was therefore suspected of having primary hyperaldosteronism. However, in two cats receiving amlodipine and one receiving both amlodipine and atenolol, ARR was within the reference range and they were consequently included in the group of cats with no evidence of hyperaldosteronism, although amlodipine might have falsely lowered the ARR. Further diagnostic efforts, including a confirmative suppression test and abdominal diagnostic imaging, would be required in these cats to establish a diagnosis of normo-aldosteronism or primary hyperaldosteronism.

The true proportion of CKD cats with primary hyperaldosteronism may also be higher than reported at present due to the effect of dehydration. Dehydration is a common consequence of the polyuria that is often associated with CKD (Polzin, 2010), and may result in elevated renin secretion. This may be especially relevant in the cats suspected of having secondary hyperaldosteronism, in which plasma concentrations of creatinine and urea were significantly higher than the other two groups, and urine SG was low. It can be hypothesized that dehydration is such a strong stimulus for renin

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secretion that it may overrule renin suppression in primary hyperaldosteronism. In addition to dehydration, intrarenal vascular changes resulting in glomerular ischemia may release renin from suppression by excessive aldosterone levels (Catena et al., 2007). Consequently, primary hyperaldosteronism may have been masked in some cats, especially those now classified as having sSHA.

On the other hand, the prevalence of ARR elevation may theoretically have been overestimated by a potentially persistent aldosterone breakthrough in some of the cats that had previously received benazepril. Aldosterone breakthrough refers to the inability of ACE inhibitor therapy to reliably suppress aldosterone secretion. A small number of studies has reported aldosterone breakthrough to occur in 40-53% of humans with chronic kidney disease that were treated with ACE inhibitors (for review, see Bomback and Klemmer, 2007), and findings by Steele and co-workers (2002) suggest that aldosterone breakthrough might occur in cats as well. Although PAC and PRA were not significantly affected in 16 hypertensive cats receiving either benazepril or enalapril, increases in PAC from baseline value occurred in individual cats at different time points shortly after initiation of therapy (Steele et al., 2002). Further studies are required to determine the prevalence of aldosterone breakthrough and the duration of its persistence following ACE inhibitor withdrawal in cats.

The ARR has very high sensitivity in humans but its specificity was only 0.61 in one study (Hirohara et al., 2001), and in a large study of patients with resistant arterial hypertension, primary hyperaldosteronism was confirmed in only about half of those with an elevated ARR (Douma et al., 2008). If the specificity of the ARR is similarly low in cats, not all CKD cats with an elevated ARR are likely to have primary hyperaldosteronism. Hence the finding of an elevated ARR should ideally be followed by one or more confirmatory tests and abdominal diagnostic imaging. With regard to diagnostic imaging it has to be noted that the findings of ultrasonography and CT of the adrenals were inaccurate in 5 of 30 reported cases of histopathologically confirmed feline primary hyperaldosteronism, failing to detect an adrenal adenoma in one cat and bilateral hyperplasia of the zona glomerulosa in four (Flood et al., 1999; MacKay et al., 1999; Bruyette, 2001; Rijnberk et al, 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005; Reimer et al., 2005; Rose et al., 2007; Renschler and Dean, 2009; Smith et al., 2012; Willi et al., 2012).

In this study, three of the seven cats with a high ARR had an elevated PAC and a PRA below or within reference range. In the remaining four cats, however, PAC was within the reference range and PRA was below or within the lower end of the reference range. Reassessment of the PRA, PAC and ARR was possible in only two of these cats. A low PRA was confirmed in one cat but not in the other. The great attention that was given to the cooling of blood samples and the freezing of plasma makes it unlikely that these low PRA values were due to temperature-related renin inactivation. We therefore interpreted the conflicting results as fluctuations in PAC and PRA, which have been demonstrated to occur in cats with primary hyperaldosteronism due to bilateral adrenocortical nodular hyperplasia (Javadi et al., 2005). The occurrence of such variation in serial ARR measurements emphasizes the need for a confirmatory test such as an oral fludrocortisone suppression test to confirm or rule out primary hyperaldosteronism in cats suspected of this disorder.

The results of this study suggest that primary hyperaldosteronism is not rare in cats with CKD. When it is confirmed, its cause should be investigated in order to determine

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the appropriate treatment. Unilateral adrenocortical adenoma or a non-metastasized adenocarcinoma should be removed surgically, if possible, whereas bilateral adrenocortical nodular hyperplasia or a metastasized adenocarcinoma require treatment with an aldosterone receptor blocker such as spironolactone.

Eliminating the source or blocking the effects of excessive aldosterone production may halt aldosterone-induced progression of CKD, as has been demonstrated in human patients with primary hyperaldosteronism (Catena et al., 2007), and may increase life expectancy and quality. We therefore conclude that testing for primary hyperaldosteronism in cats with CKD is worthwhile: it may provide treatment options to slow down the loss of kidney function and improve outcome in a potentially substantial number of patients.

Conclusion

An elevated ARR in 7 (14%) of 51 cats with CKD suggests that primary hyperaldosteronism is not uncommon in such cats. The examination for primary hyperaldosteronism is worthwhile, for its treatment may delay or halt progression of the kidney dysfunction and improve outcome. Further investigation of the prevalence of primary hyperaldosteronism in cats with chronic renal disease is warranted.

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Urinary aldosterone-to-creatinine ratio in cats before and after suppression with salt or fludrocortisone acetate

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Abstract

Background: The endocrine diagnosis of primary hyperaldosteronism in cats currently is based on an increased plasma aldosterone-to-renin ratio, which has several disadvantages for use in veterinary practice.

Objectives: To establish a reference range for the urinary aldosterone-to-creatinine ratio (UACR) and to determine whether oral administration of either sodium chloride or fludrocortisone acetate is effective for use in a suppression test.

Animals: Forty-two healthy cats from an animal shelter and one cat with primary hyperaldosteronism from a veterinary teaching hospital.

Methods: Morning urine samples for determination of the basal UACR were collected from 42 healthy cats. For the suppression tests, urine samples for the UACR were collected after twice daily oral administration for four consecutive days of either sodium chloride, 0.25 g/kg body weight (n=22) or fludrocortisone acetate, 0.05 mg/kg body weight (n=15).

Results: The median basal UACR was $7.2x10^{-9}$ (range $1.8\text{-}52.3x10^{-9}$), with a calculated reference range of $<46.5x10^{-9}$. Administration of sodium chloride resulted in adequate salt loading in 10 of 22 cats, but without significant reduction in the UACR. Administration of fludrocortisone resulted in a significant decrease in the UACR (median 78%; range 44-97%; $P<0.001$) in healthy cats. In the cat with an aldosterone-producing adrenocortical carcinoma, the basal UACR and the UACR after fludrocortisone administration were $32x10^{-9}$ and $36x10^{-9}$, respectively.

Conclusions and clinical importance: Using the UACR for an oral fludrocortisone suppression test may be useful for the diagnosis of primary hyperaldosteronism in cats.

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Introduction

Primary hyperaldosteronism has been diagnosed with increasing frequency in cats since first being reported in 1983. It may be caused by adrenocortical neoplasia or bilateral adrenocortical hyperplasia (Eger et al., 1983; Flood et al., 1999; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005). Excessive secretion of aldosterone causes increased renal reabsorption of sodium and water and increased renal excretion of potassium. These aldosterone-induced changes may result in systemic arterial hypertension and potassium depletion, signs of which can include hypokalemic paroxysmal flaccid paresis, acute blindness due to retinal detachment or intraocular hemorrhage, and other changes attributable to hypertensive damage in target organs such as the kidney, heart or brain.

The diagnosis of primary hyperaldosteronism in cats is at present mainly based upon the relation between the plasma aldosterone concentration (PAC) and plasma renin activity (PRA), i.e. an increased plasma aldosterone-to-renin ratio (ARR) (Hiramatsu et al., 1981; Javadi et al., 2004; Javadi et al., 2005). Practical disadvantages associated with determining the ARR include the large volume of blood (4 mL) that is required and the necessity to instantly freeze the plasma sample after collection. PRA measurements are time-consuming and there is a large variation in reference values among laboratories. Also, due to fluctuations in the PAC and the PRA, a single ARR result within the reference range does not exclude hyperaldosteronism and repeated measurements may be required to demonstrate this condition in cats (Javadi et al., 2005). The solution could lie in the determination of aldosterone excretion in urine, as has proved useful in humans (Brown et al., 2002). The urinary aldosterone-to-creatinine ratio (UACR) represents an integrated measure of aldosterone secretion over time. Moreover, urine for measurement of aldosterone does not require immediate freezing and can be collected quite easily.

The diagnosis of an endocrine hyperfunction frequently is based on the results of a suppression test. The suppressive agent is administered in a dose that reduces secretion of the hormone in healthy individuals, while causing little or no reduction in those affected with the disorder. In human medicine, oral or intravenous salt loading and oral administration of fludrocortisone are used in suppression tests for the diagnosis of primary hyperaldosteronism (Streeten et al., 1979; Young et al., 1988; Young, 2002; Stowasser et al., 2003).

The aims of this study were to establish a reference range for the UACR in cats and to determine whether orally administered sodium chloride or fludrocortisone acetate is effective in suppressing urinary aldosterone excretion in healthy cats.

Materials and methods

Animals

Forty-two cats from an animal shelter were enrolled in this study. The inclusion criteria were: age ≥5 months, no remarkable findings on physical examination, systemic arterial blood pressure ≤160 mmHg, plasma ARR below the upper reference limit ($<3.8 \times 10^{-9}$) (Javadi et al., 2004), and laboratory results within the following reference ranges: plasma urea concentration 6.1-12.8 mmol/L, plasma creatinine concentration 76-164 µmol/L, plasma sodium concentration (Na) 146-158 mmol/L, plasma potassium concentration (K) 3.4-5.2 mmol/L, urine specific gravity (SG) >1.020, urinary total protein-to-creatinine ratio $<10 \times 10^{-5}$, and no remarkable abnormalities in the urine sediment.

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The cats were housed and fed individually in a separate, quiet room and had access to natural light and ventilation, toys in the cages, and daily free exercise. They were groomed by and had daily social interaction with the nurses of the animal shelter. One cat was an Oriental shorthair and the other 41 were domestic shorthair. Sex and age were recorded as stated by the former owner or, in the case of stray animals, age was estimated by physical appearance, the condition of the teeth, and the presence or absence of nuclear sclerosis of the lens. The sexual integrity in female cats was judged by examining the abdominal midline for a scar indicating a previous ovariectomy or ovariohysterectomy. There were 16 intact females, 6 spayed females, 11 intact males and 9 castrated males. Their ages (known or estimated) ranged from 5 months to 9 years, with a mean of 2.6 years and a median of 2 years. Their mean body weight was 3.3 kg (median 3.2 kg; range 2.0-5.0 kg). All cats were fed a single variety of commercial canned cat food (Whiskas® beef, Mars Inc., Veghel, The Netherlands) starting at least one week before the onset of the experiments.

A privately owned, 15-year-old, male castrated Burmese cat with primary hyperaldosteronism due to a metastasized adrenocortical adenocarcinoma was also studied. The diagnosis of primary hyperaldosteronism was confirmed by the finding of a PAC of 2780 pmol/L (reference range 110-540 pmol/L) and a PRA of 270 fmol/L/s (reference range 60-630 fmol/L/s), resulting in an ARR of $10.3x10^{-9}$ (reference range $0.3\text{-}3.8x10^{-9}$). The cat's only medication at the time of diagnosis was amlodipine besylate (Norvasc®, Pfizer BV, Capelle aan den IJssel, The Netherlands) at an oral dosage of 0.2 mg/kg body weight q24h to decrease systemic arterial blood pressure.

Blood pressure measurements

Systemic arterial blood pressure was measured with an ultrasonic Doppler flow detector (Parks Model 811-B ultrasonic Doppler flow detector, Parks Medical Electronics Inc., Aloha, OR, USA) in combination with a 5 cm-wide cuff (Babyphon® infant, Rudolf Riester GmbH & Co.KG, Jungingen, Germany) and a handheld sphygmomanometer (Precisa® N, Rudolf Riester GmbH & Co.KG, Jungingen, Germany). The cats were allowed to sit or stand and were restrained as little as possible. The sphygmomanometer cuff was placed just above the right elbow and the Doppler flow detector was applied with ultrasound transmission gel (Aquasonic® 100 ultrasound gel, Parker Laboratories Inc., Fairfield, NJ, USA) to the skin just above the carpus on the medial side, from which the hair had been clipped. The probe was moved around until a clear signal was obtained from the median artery and then the cuff was gently inflated to 10-20 mmHg above the pressure at which blood flow could no longer be detected. The cuff then was slowly deflated and the pressure at which a clearly audible signal first reappeared was recorded as the systolic blood pressure. The mean value of three consecutive measurements was recorded.

UACR and suppression tests

Morning urine samples were collected at least 24 hours after completion of the initial physical and laboratory examinations for measurements of urinary aldosterone and creatinine concentrations (day 1). The UACR was the quotient of the urinary aldosterone concentration (pmol/L) divided by the urinary creatinine concentration (µmol/L).

After collection of the first urine sample, either sodium chloride or fludrocortisone acetate was administered to 40 of the 42 cats on four consecutive days (days 1-4), and the second urine sample was collected on the morning after the last dose (day 5). Tablets of sodium chloride (Natrii Chloridum 1000 mg, Genfarma BV, Zaandam, The Netherlands) were divided in half and mixed in the meals of 22 cats to provide a dosage of 0.25 g/kg body weight q12h on four consecutive days. The cats were carefully observed to insure

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that each dose was ingested completely. Morning urine samples for determination of urinary aldosterone, creatinine and Na concentrations were collected on days 1 and 5. Oral salt loading was considered successful if the urinary sodium-to-creatinine ratio (USCR) increased by at least 100%.

Eighteen cats received fludrocortisone acetate (0.0625 mg tablets; Fludrocortison®, Aesculaap BV, Boxtel, The Netherlands) at a dosage of 0.025 mg/kg body weight q12h (n=3) or 0.05 mg/kg body weight q12h (n=15) on four consecutive days. The cat with primary hyperaldosteronism also received fludrocortisone at an oral dosage of 0.05 mg/kg body weight q12h for four days. Morning samples for measurement of UACR were collected on days 1 and 5.

Blood and urine collection, sample handling and clinical biochemistry

For measurements of PAC and PRA, 4 mL blood was collected by jugular venipuncture into ice-chilled EDTA-coated tubes. Samples were centrifuged at 4°C for 12 minutes at 3500 rpm, and plasma was stored at -20°C until assayed (see below). One mL blood was collected in a heparin-coated tube for measurement of plasma urea, creatinine, Na and K concentrations at the University Veterinary Diagnostic Laboratory, Utrecht, The Netherlands. Na and K were measured with a blood gas and electrolyte analyzer (ABL, Radiometer Nederland BV, Zoetermeer, The Netherlands) and urea and creatinine were measured on a Beckman Synchron CX7 (Beckman, Mijdrecht, The Netherlands) with Beckman Coulter reagents (Beckman Coulter, Mijdrecht, The Netherlands).

Morning urine samples of 10 mL were collected from the litter boxes, which had been cleaned, dried and bedded with shredded plastic the night before. The samples were divided into two tubes. One sample was stored at -20°C for measurement of urine aldosterone and the other was used for urine SG, pH, hemoglobin, glucose, sediment, Na, creatinine and total protein. Urine SG was estimated with a refractometer (Atago SPR-T2, Atago Co., Ltd, Tokyo, Japan) and pH with indicator paper (Dual-Tint®, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Urine Na, creatinine and total protein concentrations were measured on a Beckman Synchron CX7 (Beckman, Mijdrecht, The Netherlands). Urine glucose and hemoglobin were measured with test strips (Combur-5-Test® D, Roche, Basel, Switzerland). The urinary sodium-to-creatinine ratio (USCR) was the quotient of the sodium concentration in mmol/L divided by the creatinine concentration in µmol/L. The total protein-to-creatinine ratio was the quotient of the total protein concentration in g/L divided by the creatinine concentration in µmol/L.

Hormone measurements

PAC and PRA were measured at the Department of Nephrology of the University Medical Center, Utrecht, The Netherlands, as described previously (Boer et al., 1983) and validated for the cat (Javadi et al., 2004). Briefly, for measurement of plasma renin activity, 0.5 mL of plasma was incubated at 37°C and pH 6.0 for one hour, in the presence of inhibitors of angiotensinases and angiotensin I-converting enzyme. After incubation, the samples were deproteinized with 4 mol/L acetone and ammonia (9:1 vol/vol) and centrifuged. The supernatants were evaporated and redissolved in assay buffer and angiotensin I was measured by radioimmunoassay (Antibody from Peninsula Laboratories Inc., Belmont, CA, USA; tracer from NEN Life Sciences Products, Boston, MA, USA). Aldosterone was extracted from 1 mL of plasma using dichloromethane. The extracts were evaporated and redissolved in assay buffer, and aldosterone was quantitated by radioimmunoassay (ICN Pharmaceuticals Inc., Costa Mesa, CA, USA). A similar procedure, with additional acid hydrolysis of the aldosterone-18-glucuronide binding, was applied to a 2 mL urine sample for measurement of the urine aldosterone

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concentration. For acid hydrolysis, 1 mL of a 0.2 N HCl solution was added to 0.5 mL supernatant of a urine and dichloromethane mixture (1:5 vol/vol). The sensitivity of the aldosterone assay was 10 pmol/L and urine aldosterone concentrations below the sensitivity (n=3) were set at 10 pmol/L. Pooled control samples were included in each aldosterone and renin assay. The within-assay and between-assay coefficients of variation were 8% and 15%, respectively, for the renin assay, and 6% and 14%, respectively, for the aldosterone assay. The plasma aldosterone-to-renin ratio (ARR) was the quotient of the PAC in pmol/L divided by the PRA in fmol/L/s.

The urine corticoid concentration was measured in the day 1 urine of 11 of the 15 cats that were to receive fludrocortisone acetate at a dosage of 0.05 mg/kg body weight q12h. The measurements were performed at the University Veterinary Diagnostic Laboratory, Utrecht, The Netherlands, using a radioimmunoassay as described previously (Rijnberk et al., 1988). The intra- and interassay coefficients of variation were 6% and 8%, respectively, and the sensitivity was 1 nmol/L. The urinary corticoid-to-creatinine ratio (UCCR) was the quotient of the corticoid concentration in nmol/L divided by the creatinine concentration in µmol/L.

Data analysis

Results are expressed numerically as median and range and graphically as box-and-whisker-plots. In the latter, the box represents the interquartile range from the 25th to 75th percentile, the horizontal bar through the box indicates the median, and the whiskers represent the main body of the data. Outliers are indicated by an O and extreme outliers by an asterisk.

Statistical analyses were performed using SPSS for Mac OS X (SPSS 11.0.4 for Mac® OS X, SPSS Benelux BV, Gorinchem, The Netherlands). The Kolmogorov-Smirnov test was used to test the data for normal distribution. The UACRs and USCRs before and after suppression were compared using Wilcoxon's signed-rank test. Reference ranges were determined by the non-parametric method of percentile estimates with non-parametric confidence intervals for the true percentile. $P<0.05$ was considered significant.

Results

The basal UACR in healthy cats ranged from $1.8x10^{-9}$ to $52.3x10^{-9}$, with a median of $7.2x10^{-9}$ (Figure 1) and a calculated reference range of $<46.5x10^{-9}$.

Oral NaCl administration resulted in a significant increase ($P<0.001$) in the median USCR (Figure 2). The USCR increased by 4-582% (median 103%) in 20 cats, but decreased by 19% and 29%, respectively, in 2 others.

Sodium chloride administration resulted in a twofold or higher increase in the USCR (and thereby successful salt loading) in only 10 of the 22 cats (Figure 3A). In these 10 cats, the basal UACR (median $8.4x10^{-9}$; range $3.3\text{-}52.3x10^{-9}$) did not differ significantly ($P=0.78$) from the UACR after oral salt loading (median $9.25x10^{-9}$; range $2.8\text{-}86.7x10^{-9}$) (Figure 3).

Fludrocortisone acetate at an oral dosage of 0.025 mg/kg body weight q12h in three healthy cats caused a reduction in the UACR by 23, 56 and 67%, respectively. Given orally at a dosage of 0.05 mg/kg body weight q12h in 15 healthy cats it resulted in a significant decrease ($P<0.001$) in the UACR from a median basal UACR of $6.9x10^{-9}$ (range $2.7\text{-}17.8x10^{-9}$) to a median suppressed UACR of $2.2x10^{-9}$ (range $0.9\text{-}5.4x10^{-9}$).

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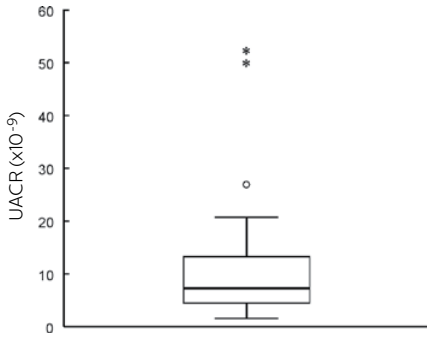

Figure 1
Basal urinary aldosterone-to-creatinine ratio (UACR) in 42 healthy cats. O: outlier; asterisk: extreme outlier.

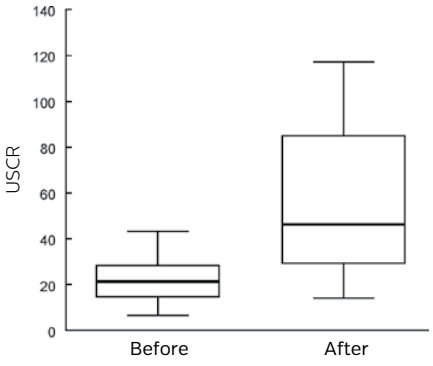

Figure 2
Urinary sodium-to-creatinine ratio (USCR) before (left) and after (right) oral administration of 0.25g sodium chloride per kg body weight q12h on four consecutive days in 22 healthy cats.

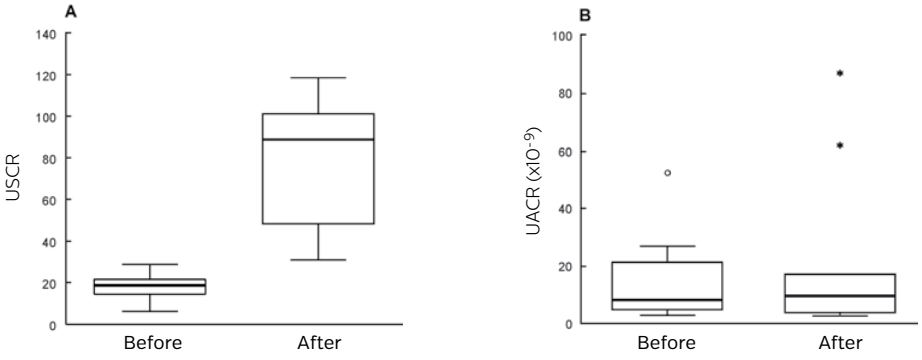

Figure 3
Urinary sodium-to-creatinine ratio (USCR, Figure 3A) and urinary aldosterone-to-creatinine ratio (UACR, Figure 3B) before and after successful oral salt loading in ten healthy cats using sodium chloride in an oral dosage of 0.25 g/kg q12h on four consecutive days. O: outlier; asterisk: extreme outlier.

The UACR after fludrocortisone administration was below $6.0x10^{-9}$ in all 15 cats. The median suppression was 78% (range 44-97%; n=15) (Figure 4). In 11 cats receiving fludrocortisone acetate orally at a dosage of 0.05 mg/kg body weight q12h the basal UCCR was within the reference range (<$42x10^{-6}$; De Lange et al., 2004). In the patient with confirmed primary hyperaldosteronism, the administration of 0.05 mg fludrocortisone per kg body weight q12h on four consecutive days was associated with an increase in the UACR from 32.3 to $36.0x10^{-9}$.

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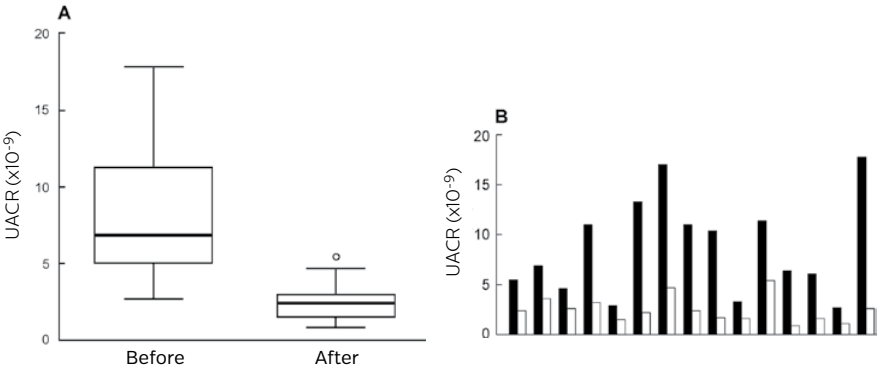

Figure 4
Urinary aldosterone-to-creatinine ratio (UACR) in 15 healthy cats, before (left) and after (right) oral administration of 0.05 mg fludrocortisone acetate per kg body weight q12h on four consecutive days. Figure 4A shows the integral data and Figure 4B shows the individual values. O: outlier.

Discussion

The main aim of this study was to determine whether a new urine-based diagnostic test, less sensitive to fluctuations in aldosterone secretion than the ARR, may be advantageous in the diagnosis of primary hyperaldosteronism in cats. Urinary aldosterone excretion relative to urine creatinine concentration was evaluated in 42 healthy cats, before and after administration of sodium chloride or fludrocortisone acetate. The calculated reference range for the basal UACR in healthy cats was $<46.5 \times 10^{-9}$. Sodium chloride administration resulted in successful oral salt loading in only 10 of 22 cats and did not significantly suppress the UACR in any. In contrast, oral administration of fludrocortisone acetate at a dosage of 0.05 mg/kg body weight q12h in 15 healthy cats resulted in a significant decrease ($P<0.001$) in the UACR from a median basal value of 6.9×10^{-9} to a median of 2.2×10^{-9}. The median suppression was 78% (range 44-97%). However, administration of 0.05 mg fludrocortisone per kg body weight q12h for four consecutive days to a cat with confirmed primary hyperaldosteronism did not suppress urinary aldosterone excretion.

The reference range for the basal UACR is relatively wide, indicating marked inter-individual variation in urinary aldosterone excretion. The UACR of 32.3×10^{-9} in the patient with confirmed primary hyperaldosteronism was within the reference range. Also, Syme et al. recently reported that the differences in basal UACR among healthy cats, cats with normotensive chronic renal failure, and cats with chronic renal failure and arterial hypertension were not significant (Syme et al., 2007). These findings illustrate that the basal UACR does not always reveal hyperaldosteronism. A suppression test may have better discriminating power.

Although an increased basal UACR may be regarded as a positive screening test, the diagnosis of primary hyperaldosteronism may require confirmation by a suppression test (Mulatero et al., 2006). Oral or IV administration of sodium chloride (salt loading) and the

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oral fludrocortisone suppression test are widely used for the diagnostic suppression of aldosterone secretion in humans suspected of primary hyperaldosteronism (Streeten et al., 1979; Young and Klee, 1988; Young, 2002; Stowasser et al., 2003). Because the aim of this study was to explore the potential of function tests that are practical, non-invasive and easy to perform in veterinary practice, attention was given to the suppressive effects of orally administered sodium chloride and fludrocortisone acetate.

Sodium chloride was mixed into a commercial canned cat food. Although the full dose of sodium chloride was ingested in all cases, oral salt loading was successful (i.e. causing a twofold or higher increase in USCR) in only 10 of 22 cats. In these cats, aldosterone excretion was not significantly suppressed. Cats likely excrete an excessive sodium chloride load rapidly, and consequently the effect on aldosterone secretion could no longer be detected in urine collected the morning after the last evening dose of sodium chloride. Although 24-hour urine collection would identify the sodium and aldosterone excretion after sodium chloride loading, full 24-hour urine collection is not a realistic, practical option in most cats. Therefore, based on the results of this study, it does not appear that oral salt loading is a useful test for cats suspected of primary hyperaldosteronism.

Fludrocortisone administered orally in a dosage of 0.025 mg/kg body weight q12h to three cats caused urinary aldosterone excretion to decrease by 23%, 56% and 67%, respectively. In view of the small decrease in one cat, the dose was doubled in the remaining 15 cats in this study. In all of them, the oral dosage of 0.05 mg/kg body weight q12h had a marked effect on urinary aldosterone concentration. The UACR was decreased significantly, by a minimum of 44% and a median of 78%. In contrast, oral administration of fludrocortisone in the same dose to the cat with confirmed primary hyperaldosteronism was followed by an increase rather than a decrease in the UACR. Although limited to a single case, the magnitude of the difference suggests that this suppression test may prove useful in the diagnosis of primary hyperaldosteronism in cats.

The main application of the fludrocortisone suppression test is in cats suspected of primary hyperaldosteronism. Because fludrocortisone acetate potentially can have adverse effects on systemic arterial blood pressure and plasma potassium concentration in patients already prone to systemic arterial hypertension or hypokalemia, it is essential to monitor patients for these adverse effects.

The reference group differed from cats with primary hyperaldosteronism in at least two respects: age distribution and environmental circumstances. Primary hyperaldosteronism has been reported in cats ≥6 years of age (Eger et al., 1983; Flood et al., 1999; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005). Although the median age of the cats in this study was 2 years, the median plasma aldosterone concentration in cats does not differ significantly among age groups (Javadi et al., 2004), and thus the age mismatch may not be relevant.

Basal levels of stress presumably are different between cats in an animal shelter and those in private homes (the source of patient populations). ACTH release in response to stress may enhance the secretion of both aldosterone and cortisol. The effect of stress on cortisol secretion was illustrated in a study showing that the UCCR was increased in 12 of 97 cats in an animal shelter (McCobb et al., 2005). Aldosterone secretion is mainly regulated by angiotensin II and plasma K concentration. An acute increase of plasma

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ACTH concentration also leads to temporary stimulation of aldosterone secretion (McDougall et al., 1980; Braley et al., 1992). However, in cats that were likely to have been acutely stressed by transport, handling and sampling by venipuncture, Javadi et al. found that plasma ACTH concentration was correlated positively with plasma cortisol concentration, but not with plasma aldosterone concentration (Javadi et al., 2004). Thus, in the study reported here the influence of ACTH-induced aldosterone release on the UACR can be expected to have been small. Moreover, McCobb et al. noted that the UCCR was lower in cats housed in modern, enriched, animal shelters than in cats housed in traditional animal shelters (McCobb et al., 2005). The animal shelter in this study was comparable to the modern, enriched, animal shelters described by McCobb et al., implying that stress responses were probably not as extreme as reported for some of the cats in traditional animal shelters. This was confirmed by the fact that the UCCR was within the reference range in all 11 cats in which this variable was measured.

Shredded plastic was used as litter box bedding for urine collection. In an earlier study, non-absorbent litter box material did not affect the UCCR (H.S. Kooistra, unpublished observation). Since aldosterone is structurally related to cortisol, it was assumed that this bedding material would not significantly influence the UACR either. Finding a major, fludrocortisone-induced decline in the UACR from baseline levels in all healthy cats, but not in the cat with confirmed primary hyperaldosteronism, further supported the assumption that shredded plastic is a suitable cat box filler for urine collection for the UACR.

At the time the aldosterone assays for this study were performed, acid hydrolysis was included routinely in order to free aldosterone from its 18-glucuronide binding. In a recent study by Syme et al., however, acid hydrolysis did not lead to significant increases in aldosterone recovery from feline urine (Syme et al., 2007). From the latter study it may be concluded that acid hydrolysis probably is not an essential step in aldosterone measurement in feline urine.

In conclusion, measurement of the UACR is a practical, non-invasive method which, combined with fludrocortisone-induced suppression, may be a useful tool in the diagnosis of primary hyperaldosteronism in cats. Administration of fludrocortisone acetate caused a significant reduction in the UACR in healthy cats but not in a cat with confirmed primary hyperaldosteronism.

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Evaluation of the oral fludrocortisone suppression test for diagnosing primary hyperaldosteronism in cats

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Abstract

Background: Primary hyperaldosteronism (PHA) in cats is suggested by clinical signs and an elevated plasma aldosterone-to-renin ratio (ARR), but a test to confirm the diagnosis is lacking.

Hypothesis: Fludrocortisone does not suppress urinary aldosterone excretion in cats with PHA, but does so in cats with arterial hypertension due to other causes.

Animals: Nineteen client-owned cats with arterial hypertension due to PHA (n=9) or other causes (n=10).

Methods: Prospective clinical study. The urinary aldosterone-to-creatinine ratio (UACR) was determined in morning urine before, during and after four days of oral fludrocortisone administration in a dose of 0.05 mg/kg q12h. Arterial blood pressure and plasma potassium concentration were measured before and after fludrocortisone administration.

Results: A basal UACR above 46.5×10^{-9}, the upper limit of the reference range, was found in three cats with PHA. All PHA cats had basal UACRs $>7.5 \times 10^{-9}$. In all non-PHA cats with a basal UACR $>7.5 \times 10^{-9}$, fludrocortisone administration induced >50% suppression. In contrast, fludrocortisone administration resulted in <50% suppression in six of the nine PHA cats. Neither basal UACR, nor UACR following suppression testing, correlated with the etiology of PHA (adenoma, adenocarcinoma or suspected bilateral hyperplasia of the zona glomerulosa). Fludrocortisone induced hypokalemia in seven cats, but did not induce or exacerbate arterial hypertension.

Conclusions and clinical importance: Measuring the UACR before and after four days of administering fludrocortisone is a practical method of confirming most cases of PHA in cats, and of substantiating the absence of PHA in cats having an ARR within the reference range.

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Introduction

Primary hyperaldosteronism (PHA), also termed primary aldosteronism, low-renin hyperaldosteronism, or Conn's syndrome, is the adrenocortical disorder of autonomous hypersecretion of aldosterone (Galac et al., 2010). In 1983, nearly 30 years after the first reported case in humans (Conn, 1955), PHA was first reported to have occurred in a cat (Eger et al., 1983). PHA in cats was initially thought to be rare but the number of reports has risen considerably in the past 15 years (Flood et al., 1999; MacKay et al., 1999; Maggio et al., 2000; Moore et al., 2000; Bruyette, 2001; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005; Rose et al., 2007; Briscoe et al., 2009; Renschler and Dean, 2009; Djajadiningrat-Laanen et al., 2011). Increased awareness of the disease will probably lead to a further increase in recognized cases.

The mineralocorticoid excess in cats with PHA, originating from unilateral or bilateral neoplasia or bilateral hyperplasia of the adrenal zona glomerulosa, can result in systemic arterial hypertension, hypokalemia, or both, and has also been associated with progressive loss of kidney function (Javadi et al., 2005). PHA can be treated surgically or pharmacologically, and hence the hypertension and hypokalemia might be cured or alleviated and the deterioration of kidney function might be retarded. It is thus worth evaluating aldosterone secretion in cats with arterial hypertension, hypokalemia or chronic kidney disease.

The diagnosis of PHA in cats is currently based on the history, clinical signs, routine laboratory results, and the use of an elevated plasma aldosterone-to-renin ratio (ARR) as a positive screening test. Ideally, the autonomous hypersecretion of aldosterone should be confirmed by a suppression test. In the absence of such a test for PHA in cats, diagnostic imaging is usually employed to detect abnormalities of size or structure of the adrenal gland(s) suggesting adrenal neoplasia and to detect any distant metastases. However, ultrasonographic and CT examinations failed to detect an adrenal adenoma and bilateral hyperplasia of the zona glomerulosa in 4 of 21 cats with histopathologically confirmed PHA (Flood et al., 1999; MacKay et al., 1999; Bruyette, 2001; Rijnberk et al., 2001; Ash et al., 2005; DeClue et al., 2005; Javadi et al., 2005; Rose et al., 2007; Renschler and Dean, 2009). The inaccuracy of diagnostic imaging and the lack of other practicable diagnostic methods underscore the need for a reliable confirmatory test for PHA in cats.

In the fludrocortisone suppression test, the mineralocorticoid fludrocortisone promotes sodium and water retention, and thereby induces blood volume expansion. In cats with normal aldosterone regulation this should lead to suppressed renin and aldosterone release. In contrast, cats that are refractory to normal aldosterone regulation expectedly demonstrate a lack of aldosterone suppression. Fludrocortisone suppression of urinary aldosterone excretion was investigated in 15 healthy cats and one cat with a confirmed aldosterone-secreting adrenocortical carcinoma (Djajadiningrat-Laanen et al., 2008). The basal urinary aldosterone-to-creatinine ratio (UACR) in the cat with PHA did not exceed the wide reference range in healthy cats, while fludrocortisone given orally for four days in a dose of 0.05 mg/kg body weight q12h caused a significant decrease in the UACR in healthy cats, but no decrease in the cat with PHA. The fludrocortisone suppression test was therefore considered promising.

The aim of this study was to evaluate the efficacy and safety of the oral fludrocortisone suppression test to confirm the diagnosis of PHA in cats with arterial hypertension or hypokalemia and arterial hypertension. Changes in urinary aldosterone

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excretion were monitored from day to day to determine the minimum duration of the test. Side effects, such as a transient decrease in the plasma potassium concentration or a rise in arterial blood pressure, were also documented.

Materials and Methods

Animals

Nineteen client-owned cats presented with arterial hypertension (n=10) or hypokalemia and arterial hypertension (n=9) were enrolled in this prospective clinical study, with the informed consent of their owners. Based on the plasma aldosterone-to-renin ratio (ARR), the arterial hypertension or hypokalemia and arterial hypertension were attributed to primary hyperaldosteronism in nine cats (PHA group) and other causes in ten cats (non-PHA group). Sixteen cats were treated at the Utrecht University Clinic for Companion Animals, The Netherlands. Urine samples before and after suppression were also included from two cats referred to the Vetsuisse Faculty of the University of Zurich, Switzerland, and from one cat referred to the Faculty of Veterinary Medicine of the Aristotle University of Thessaloniki, Greece. In these three cats the diagnosis of PHA was based on increased PAC and suppressed PRA values, but the values were not included in statistical analyses because they were determined in other laboratories. The results of routine laboratory examinations and systolic blood pressure measurements in these three cats were excluded from statistical analysis for the same reason.

The PHA group consisted of six neutered females, two castrated males and one intact male, with a median age of 13 years (range 8-19 years, n=8; the age of one adopted stray cat was unknown). Eight of the PHA group were domestic shorthair cats and one was a Burmese. At admission, median systolic blood pressure was 193 mmHg (range 160-280 mmHg) and median plasma potassium concentration was 3.1 mmol/L (range 1.8-4.3 mmol/L). All nine cats were hypertensive, despite antihypertensive medication in one, and five were hypokalemic, with associated muscle weakness in three, despite oral potassium supplementation in two. Abdominal ultrasonography revealed an adrenal mass in seven of the PHA cats and normal-sized adrenal glands in two. Cytological examination of a fine-needle aspiration biopsy in a PHA cat with a unilateral adrenal mass and radiographic findings suggestive of pulmonary metastases indicated an adrenal adenocarcinoma. Histopathological examination in four different PHA cats revealed an adrenal adenocarcinoma in one and an adrenal adenoma in three, one of which was designated multinodular.

The non-PHA group consisted of four neutered females and six castrated males, with a median age of 14 years (range 11-16 years). There were five domestic shorthair cats, two British shorthairs, one Persian, and two cross-breds. The median systolic blood pressure at admission was 225 mmHg (range 166-283 mmHg) and median plasma potassium concentration was 3.6 mmol/L (range 2.8-4.1 mmol/L). All of the non-PHA cats were hypertensive, despite antihypertensive medication in three, and four were hypokalemic, one with muscle weakness despite oral potassium supplementation. Abdominal ultrasonography in the nine cats revealed an adrenal mass in one, and post-mortem examination showed this to be a benign adrenocortical mass which was immunonegative for neurospecific enolase (Javadi et al., 2005). Combined with the unremarkable ARR, these findings are consistent with a non-aldosterone-secreting adrenal mass.

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Prior to the fludrocortisone suppression test, an attempt was made to normalize blood pressure with oral amlodipine in eight PHA and eight non-PHA cats. One PHA cat and two non-PHA cats also received atenolol. Potassium chloride or potassium gluconate was added to the food as required according to the plasma potassium concentration. Other oral medications included benazepril in three cats, spironolactone in one other, and methimazole in another. Prednisolone was administered to one cat to alleviate neurological signs that were found at post-mortem examination to have been caused by a meningioma. The medications were continued during the fludrocortisone suppression test, with two exceptions: benazepril was withheld for seven days prior to the test in one non-PHA cat, and both amlodipine and benazepril were withheld for two days before the test in a cat with PHA. Another hypokalemic PHA cat received additional intravenous potassium supplementation during the test.

Systolic blood pressure measurements and ophthalmic examination

Arterial blood pressure measurements were performed before any other examination, after a ten-minute acclimatization, using a Doppler flow detector (Parks Model 811-B ultrasonic Doppler flow detector, Parks Medical Electronics Inc., Aloha, OR, USA), a 5 cm-wide cuff (Babyphon® infant, Rudolf Riester GmbH & Co.KG, Jungingen, Germany), and a handheld sphygmomanometer (Precisa® N, Rudolf Riester GmbH & Co.KG, Jungingen, Germany). The mean value of at least three consecutive measurements with less than 20% variation was used. Arterial hypertension was diagnosed if the mean systolic blood pressure was >180 mmHg, or was >160 mmHg together with hemorrhage in the anterior chamber, vitreous, retina or subretinal space, retinal vascular tortuosity, multifocal to complete retinal detachment, or any combination of these signs. The ophthalmic examination was performed in a darkened room using a slit lamp microscope (Kowa SL-15, Kowa Europe GmbH, Düsseldorf, Germany) and an indirect ophthalmoscope (Heine Video OMEGA® 2C, Heine Optotechnik, Herrsching, Germany).

Blood and urine sampling and examination

An 8 mL blood sample was obtained by jugular venipuncture for routine laboratory examination and for measurement of PAC and PRA. For the latter measurements, blood was collected into an ice-chilled EDTA-coated tube and kept on ice until centrifuging. The sample was centrifuged for 10 minutes at 3000 rpm and plasma was stored at -20°C until further processing. PAC and PRA were measured as described previously (Boer et al., 1983), and validated for the cat (Javadi et al., 2004). The plasma aldosterone-to-renin ratio (ARR) was calculated by dividing the PAC (pmol/L) by the PRA (fmol/L/s). PRA values below the detection limit were set at 40 fmol/L/s in order to allow calculation of the minimum ARR.

Morning urine samples were collected by the owner from the cat's litter box, which had been cleaned, dried and bedded with a non-absorbent cat litter (Katkor®, Reinvet Products, Utrecht, The Netherlands), and were kept refrigerated until processed. A sample for routine urine examination was collected within 24 hours prior to the examination, or obtained by cystocentesis. Urinary aldosterone concentration was measured as described previously (Djajadiningrat-Laanen et al., 2008). The urinary aldosterone-to-creatinine ratio (UACR) was calculated by dividing the urinary aldosterone concentration (pmol/L) by the urinary creatinine concentration (µmol/L). The percentage suppression of the UACR by fludrocortisone was calculated as 100 x (UACR on day 0 – UACR on day X) / UACR on day 0.

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Adrenal ultrasonography

Abdominal ultrasonography was performed with a high-definition digital ultrasound system (ATL Ultramark HDI 3000, Philips, Eindhoven, The Netherlands; HD11 XE, Philips, Eindhoven, The Netherlands) by use of a 7.5 MHz phased-array transducer or an 8.5 MHz broadband curved-array transducer, or both.

Oral fludrocortisone suppression test

Arterial blood pressure and plasma potassium concentration were measured at a median interval of seven days (range 3-10 days) before the suppression test. Morning urine samples were collected for measurement of the UACR. After collection of the first urine sample (day 0), fludrocortisone acetate (Florinef® acetate, Bristol-Myers Squibb BV, Woerden, The Netherlands) was administered in a dose of 0.05 mg/kg body weight orally q12h for four and a half days (days 0-4). The suppression test was performed at home in all Dutch cats and one Swiss cat, and at the respective university clinics in the other Swiss cat and the Greek cat. Urine samples for determination of the UACR were collected every morning (days 1-4) or, in four cases, on the morning following the last evening dose only (day 4). After collection of the last urine sample, the last morning dose of fludrocortisone was administered to ten non-PHA cats and five PHA cats, and the arterial blood pressure and plasma potassium concentration were measured at about the same time of day as before the suppression test.

Statistics

Statistical analyses were performed using IBM® SPSS® Statistics version 19.0.0 (IBM® Corporation, Armonk, NY, USA). The Shapiro-Wilk test was used to test the data for normal distribution. Groups were compared using Levene's test for equality of variances and an independent-samples t-test for equality of means for data with a normal distribution, and the Wilcoxon signed-rank test for data with a non-Gaussian distribution. $P<0.05$ was considered significant. Data are expressed as median and range.

Parameter (units)	Non-PHA	PHA	Reference range
Sodium (mmol/L)	150.5 (145-153) [10]	151 (147-155) [6]	146-158
Potassium (mmol/L)	3.65 (3.1-4.6) [10]	2.85 (1.8-3.7) [6]	3.4-5.2
Urea (mmol/L)	11.2 (8.8-14.7) [10]	13 (7.5-16.2) [6]	6.1-12.8
Creatinine (µmol/L)	161 (113-227) [10]	157.5 (100-198) [6]	76-164
Calcium (mmol/L)	2.575 (2.43-2.83) [8]	2.625 (2.38-2.70) [4]	2.36-2.86
Phosphate (mmol/L)	1.2 (0.99-1.66) [9]	1.15 (1.05-1.37) [4]	0.89-2.05
Albumin (g/L)	27 (22-40) [8]	25 (22-26) [3]	25-34
Total thyroxine (nmol/L)	21 (16-56) [9]	17 (14-38) [4]	15-45
Fructosamine (µmol/L)	204 (166-241) [9]	188 (179-303) [5]	156-240
Hematocrit (L/L)	0.34 (0.27-0.38) [10]	0.36 (0.35-0.38) [3]	0.28-0.47
Urinalysis			
Specific gravity	1.030 (1.020-1.046) [10]	1.025 (1.016-1.040) [5]	>1.020
Protein-to-creatinine ratio	0.24 (0.1-0.95) [10]	0.19 (0.1-0.25) [4]	<0.4

Table 1
Findings on routine blood and urine examination in 19 cats with arterial hypertension or hypokalemia and arterial hypertension due to primary hyperaldosteronism (PHA, nine cats) or other causes (non-PHA, ten cats). Data are presented as median and range (in brackets). The number of cats on which the values are based is indicated [in square brackets].

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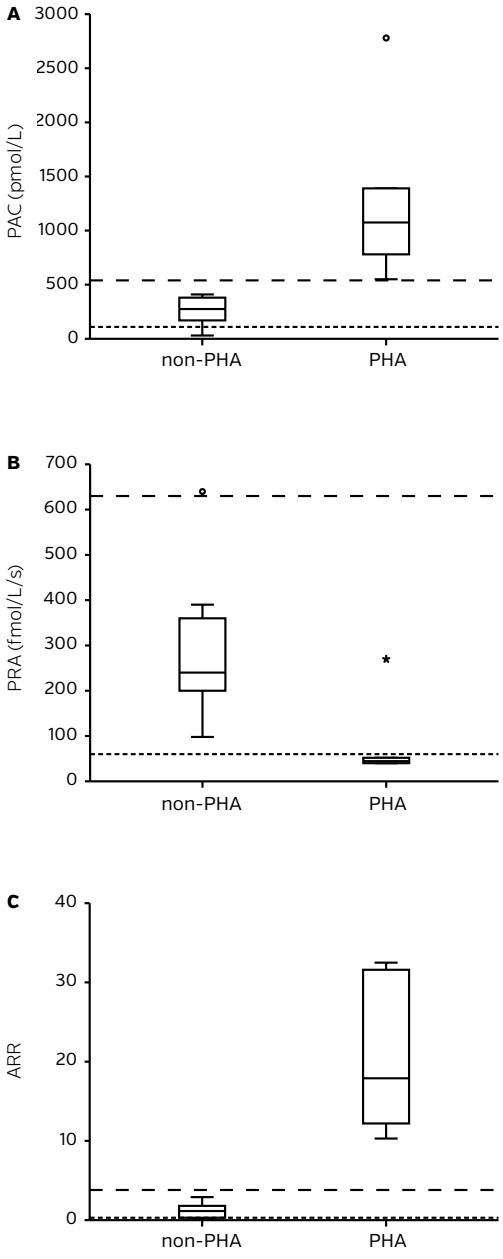

Figure 1
Plasma aldosterone concentration (PAC) (A), plasma renin activity (PRA) (B), and aldosterone-to-renin ratio (ARR) (C) in 16 cats with arterial hypertension or hypokalemia and arterial hypertension due to primary hyperaldosteronism (PHA, six cats) or other causes (non-PHA, ten cats). o indicates an outlier and * indicates an extreme outlier. The upper and lower limits of the reference ranges are indicated by a coarse and a fine dotted line, respectively.

Results

PAC and ARR were increased in all PHA cats and PRA was suppressed in all but one PHA cat, whereas these parameters were within or near the limit of the reference range in non-PHA cats (Figure 1). The plasma potassium concentration in PHA cats was significantly lower (P=0.019) than that in non-PHA cats, while other parameters did not differ significantly between these two groups (Table 1).

The basal UACR was significantly higher (P<0.01) in the PHA cats than in the non-PHA cats (Table 2). There was a significant difference (P<0.01) between UACR before and after fludrocortisone administration in the non-PHA cats, but not in the PHA cats.

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	UACR (x10^{-9})		Systolic blood pressure (mmHg)		Plasma potassium concentration (mmol/L)	
Time	Before	After	Before	After	Before	After
Non-PHA	6.25 (2.5-17.2)	2.8 (0.7-7.3)	149 (130-283) [8]	146 (126-263) [8]	3.8 (3.3-4.6) [9]	3.3 (2.7-4.9) [9]
PHA	18.2 (10.6-135)	20.9 (4.7-156)	141 (122-151) [3]	142 (123-144) [3]	3.45 (3.1-3.6) [4]	3.25 (2.6-4.1) [4]
Reference range	<46.5	<6.0	<160		3.4-5.2	

Table 2
Median urinary aldosterone-to-creatinine ratio (UACR), systolic blood pressure, and plasma potassium concentration before and after oral administration of fludrocortisone acetate in a dose of 0.05 mg/kg q12h for four consecutive days, to 19 cats with systemic arterial hypertension or hypokalemia and systemic arterial hypertension related to primary hyperaldosteronism (PHA, nine cats) or other causes (non-PHA, ten cats). The numbers of cats on which the values for systolic blood pressure and plasma potassium concentration are based are indicated [in square brackets].

In all non-PHA cats the basal UACR was within the reference range of <46.5x10^{-9}, whereas the basal UACR exceeded the reference range in three PHA cats (Figure 2). All PHA cats had basal UACRs >7.5x10^{-9}. After four days of fludrocortisone administration, the UACR was suppressed in all non-PHA cats by a median of 62.5% (range 33-76%). In contrast, the UACR was suppressed in only four of the nine PHA cats, at a maximum of 70%. The suppression in the non-PHA cats did not differ significantly (P=0.11) from the median suppression of 78% (range 44-97%) reported in 15 healthy cats (Djajadiningrat-Laanen et al., 2008). Also, the suppressed UACR values on day 4 in non-PHA cats were not significantly different (P=0.39) from those reported in healthy cats (Djajadiningrat-Laanen et al., 2008).

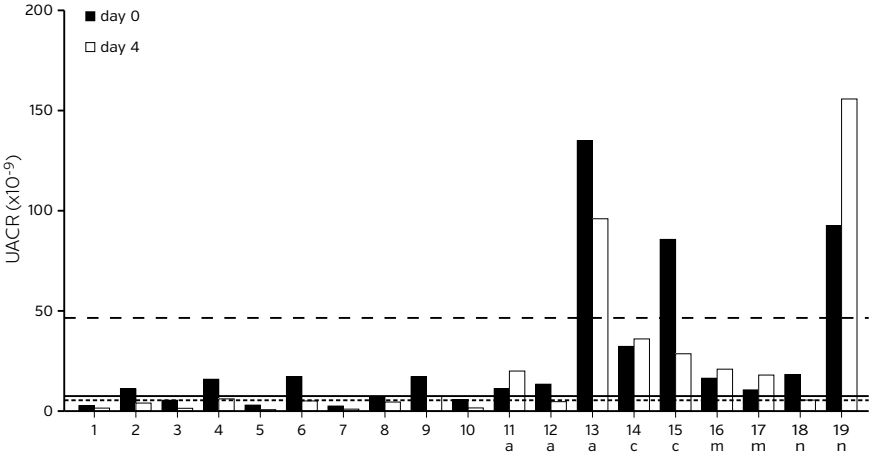

Figure 2
Urinary aldosterone-to-creatinine ratio (UACR) before (day 0) and after (day 4) the oral administration of fludrocortisone acetate at 0.05 mg/kg q12h for four consecutive days to 19 cats with arterial hypertension or hypokalemia and arterial hypertension, related to primary hyperaldosteronism (case numbers 11-19) or other causes (case numbers 1-10). The coarse dotted line represents the upper limit of the reference range for the basal UACR (day 0) in healthy cats (Djajadiningrat-Laanen et al., 2008), and the fine dotted line the maximum UACR on day 4 in healthy cats (Djajadiningrat-Laanen et al., 2008). The solid line corresponds to a UACR of 7.5x10^{-9}. Primary hyperaldosteronism was associated with (a) adrenocortical adenoma, (c) adrenocortical carcinoma, (m) adrenal mass on ultrasonographic examination, and (n) normal-sized adrenal glands on ultrasonographic examination.

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Suppression of the UACR by fludrocortisone was not related to the etiology of PHA. The UACR was suppressed in two cats with adrenocortical adenoma but increased in the third, suppressed in only one of two cats with an adrenocortical carcinoma, and suppressed in only one of two cats with PHA and normal-sized adrenal glands by ultrasonography, suggestive of bilateral hyperplasia of the zona glomerulosa.

In most non-PHA cats the UACR was increased on individual days of fludrocortisone administration before eventually being suppressed (Figure 3). In comparison with the non-PHA cats, the five with PHA had only mild fluctuations in day-to-day suppression.

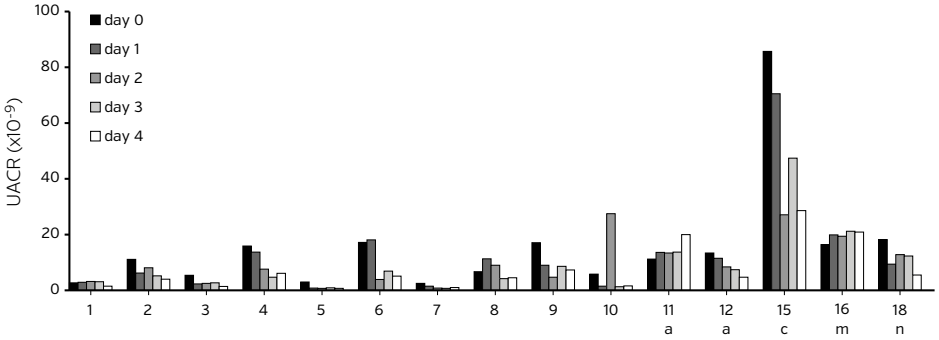

Figure 3

Urinary aldosterone-to-creatinine ratio (UACR) on days 0-4 of oral fludrocortisone administration at 0.05 mg/kg q12h to 15 cats with arterial hypertension or hypokalemia and arterial hypertension, related to primary hyperaldosteronism (PHA, case numbers 11-18) or other causes (non-PHA, case numbers 1-10). Primary hyperaldosteronism was associated with: (a) adrenocortical adenoma, (c) adrenocortical carcinoma, (m) adrenal mass on ultrasonographic examination, and (n) normal-sized adrenal glands on ultrasonographic examination.

Valid measurements of pre- and post-suppression systolic blood pressure were obtained in eight non-PHA and three PHA cats, and measurements of plasma potassium concentration in nine non-PHA and four PHA cats (Table 2). Neither systolic blood pressure nor plasma potassium concentration before and after fludrocortisone suppression differed significantly in either group. Any increments in systolic pressure during the fludrocortisone suppression test were <10% of the pretest value, and did not result in systolic arterial pressures >160 mmHg. Plasma potassium concentration decreased in six non-PHA and two PHA cats, by a median of 0.65 mmol/L (range 0.5-1.7 mmol/L), and reached values below the reference range in seven cats. This was associated with muscle weakness in one PHA cat.

Discussion

Basal UACR values in cats with PHA overlapped with those in cats with arterial hypertension or hypokalemia and arterial hypertension due to other causes, but basal UACR was >7.5x10^{-9} in all cats with PHA and above the reference range only in individual cats with PHA. In other words, an elevated basal UACR pointed to PHA and a basal UACR <7.5x10^{-9} excluded PHA. For UACR values between 7.5x10^{-9} and the upper limit of the reference range, the fludrocortisone suppression test was required for differentiation. As in healthy cats, four days of oral fludrocortisone administration induced >50% suppression in all non-PHA cats with basal UACRs >7.5x10^{-9}. In contrast, fludrocortisone administration resulted in <50% suppression in six of the nine PHA cats. Applying the criteria for both basal UACR and fludrocortisone suppression test results – i.e. a basal UACR <7.5x10^{-9} excludes PHA, a basal UACR above the reference range points to PHA

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and, in cats with a basal UACR between $7.5x10^{-9}$ and $46.5x10^{-9}$, >50% suppression of the UACR excludes PHA – correctly indicated non-PHA in all cats in which ARR was not elevated and correctly indicated PHA in seven of the nine cats in which ARR was elevated.

Multinodular disease of the zona glomerulosa was confirmed in one and suspected in one other PHA cat with a basal UACR within the reference range and a >50% suppression after fludrocortisone administration. The former had a unilateral multinodular adrenocortical adenoma confirmed histologically and the latter had adrenals of normal size by ultrasonography, suggesting that bilateral hyperplasia of the zona glomerulosa caused the elevated ARR. Multinodular disease of the zona glomerulosa might have rendered these cats still partially susceptible to normal aldosterone regulation, which could explain the fludrocortisone suppression test results. In another PHA cat with normal-sized adrenals on ultrasonography, however, the basal UACR was elevated and not suppressed by fludrocortisone. These three cases support the impression of considerable individual variation in both the level and the autonomy of aldosterone secretion in cats with multinodular disease of the zona glomerulosa (Javadi et al., 2005).

Considerable individual variation in both the basal UACR and suppression was found in cases of zona glomerulosa neoplasia. This indicates that neither an elevated basal UACR nor a specific level of suppression can be used to predict the etiology (adenoma, adenocarcinoma, or suspected bilateral hyperplasia of the zona glomerulosa) of PHA.

Mild day-to-day fluctuations in UACR, presumably mirroring daily fluctuations in aldosterone secretion, were noted in almost all cats throughout the fludrocortisone suppression test. The nearly fivefold increase in the UACR in one cat on day 2 of fludrocortisone administration is unexplained.

Fludrocortisone can have side effects due to activation of mineralocorticoid receptors in the distal nephron. Enhanced sodium and water resorption and potassium excretion can potentially lead to arterial hypertension, hypokalemia, or both. This could be of concern in cats prone to, or affected by, arterial hypertension or hypokalemia. In seven of the eleven cats in which systolic blood pressure was measured before and after the suppression test, fludrocortisone had little or no effect. The small changes observed probably represented normal fluctuations. Changes in plasma potassium concentration were also mild in most of the cats but decreases >1 mmol/L did occur in three and hypokalemia was induced or exacerbated in seven cats, leading to muscle weakness in one. Daily measurements of plasma potassium are therefore advisable to optimize potassium supplementation during the test period.

The minimum duration of the fludrocortisone suppression test can be derived from Figure 3. Maximum suppression of UACR was achieved after a minimum of three days in nine of the ten non-PHA cats, but in one cat marked suppression did not occur until day 4. Hence a duration of four days would seem advisable, as is used in humans (Funder et al., 2008).

Guidelines for the test in humans include insuring that the patients are potassium-replete and that medications that markedly affect the ARR, such as spironolactone, are discontinued for at least four weeks (Funder et al., 2008). Further, if hypertension can be controlled with relatively non-interfering medications such as non-dihydropyridine calcium channel antagonists and alpha-adrenergic blockers, it is also advisable to withdraw beta-adrenergic blockers, dihydropyridine calcium channel antagonists,

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and angiotensin-converting enzyme inhibitors for at least two weeks prior to testing, although these guidelines are currently under debate (Solar et al., 2012). Unfortunately, most cats with PHA have severe arterial hypertension, and this is best controlled using a dihydropyridine calcium channel antagonist (e.g. amlodipine), either alone or in combination with a beta-adrenergic blocker or an angiotensin-converting enzyme inhibitor (Brown et al., 2007). Although these guidelines were followed in two initial cats of our study (one of which was already permanently blind due to complications of arterial hypertension), it was then decided to aim for a stable arterial pressure within the reference range before starting the test, in order to preserve or try to restore vision, and reduce the risk of further hypertension-induced damage to organs such as the heart, kidneys and brain. Therefore most cats of our study received amlodipine, and some also received atenolol. Although it is unknown whether these medications affected suppression test results, any such effects should have occurred in both PHA and non-PHA cats.

Spironolactone was considered essential during the fludrocortisone suppression test in a cat with an adrenocortical adenoma, in which normokalemia could not be achieved by oral potassium supplements alone. The short-term administration of spironolactone is unlikely to have contributed to the extremely high basal UACR of $135x10^{-9}$ found in this cat, but might have falsely lowered the suppression rate by competing with fludrocortisone for the mineralocorticoid receptor.

A confirmative test should be safe and practical in order to facilitate its wide application. A test based on suppression of urinary aldosterone excretion rather than ARR achieves this, since urine can be collected easily and non-invasively, and aldosterone is sufficiently stable to allow sample shipping without the temperature constraints for PRA preservation. The aldosterone level in a morning urine sample also reflects aldosterone secretion over a long interval rather than at a single point in time. The UACR before and after suppression might even be an alternative to the ARR in situations in which PRA measurement is not practicable.

In summary, primary hyperaldosteronism should be considered in any cat with hypokalemia, arterial hypertension, chronic kidney disease, or all, and other potential causes should be excluded. Following a positive screening test, i.e. an elevated ARR, the oral fludrocortisone suppression test can be used to confirm the diagnosis. Diagnostic imaging techniques such as ultrasonography and computed tomography should be used to determine the laterality of the excessive aldosterone production.

Conclusion

The oral fludrocortisone suppression test appears to be reliable to exclude the diagnosis of PHA in cats with an ARR within the reference range. In addition, it confirms most cases of PHA, although it does not identify all those with multinodular disease of the zona glomerulosa. Our findings suggest that the fludrocortisone suppression test should be performed in cats with a basal UACR between $7.5x10^{-9}$ and $46.5x10^{-9}$, and that suppression <50% indicates inappropriate aldosterone secretion. Neither an elevated basal UACR nor a specific level of suppression can be used to predict the etiology (adenoma, adenocarcinoma, or bilateral hyperplasia of the zona glomerulosa) of PHA. Arterial normotension and normokalemia should be established before the fludrocortisone suppression test is undertaken and it can be necessary to monitor plasma potassium concentration during the test.

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07

Urinary aldosterone-to-creatinine ratio after fludrocortisone suppression, consistent with primary hyperaldosteronism, in a cat

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Abstract

A 9-year-old cat was investigated because of clinical signs and laboratory abnormalities attributed to arterial hypertension (mean systolic arterial pressure: 290 mmHg). Plasma aldosterone concentration (PAC) was increased on admission (651 pmol/L), while serum creatinine and potassium concentrations were within the reference range. A second increased PAC (879 pmol/L) and normal plasma renin activity (1.85 ng/mL/h) resulted in an increased aldosterone-to-renin ratio, which was suggestive of primary hyperaldosteronism. In order to further support the diagnosis of primary hyperaldosteronism, the urinary aldosterone-to-creatinine ratio (UACR) was calculated, before and after oral administration of fludrocortisone acetate (0.05 mg/kg q12h for four consecutive days). UACR was 92.6×10^{-9} before fludrocortisone administration, and 155.8×10^{-9} four days later. Absence of suppression was typical of primary hyperaldosteronism. The cat had a limited response to antihypertensive medication and died before treatment for primary hyperaldosteronism could be instituted. Necropsy was not allowed by the owner.

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Introduction

Feline primary hyperaldosteronism is a rarely diagnosed endocrinopathy, mainly caused by unilateral or bilateral adrenal micro- and macroadenomas or micronodular hyperplasia of the zona glomerulosa, with hypokalemia and systemic hypertension as its laboratory and clinical hallmarks (Djajadiningrat-Laanen et al., 2011). Its diagnosis is usually straightforward, even though chronic renal failure and activation of the renin-angiotensin-aldosterone system may mimic its clinicopathological abnormalities. In order to differentiate primary from secondary hyperaldosteronism, the use of a dynamic test, such as the urinary aldosterone-to-creatinine ratio (UACR) before and after suppression with fludrocortisone acetate, has recently been suggested as an alternative to the aldosterone-to-renin ratio (ARR) (Djajadiningrat-Laanen et al., 2008). To our knowledge, this is the second reported case in which this test has been performed.

Case report

A neutered male, 9-year-old, domestic shorthaired cat was admitted to the Companion Animal Clinic of the Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, because of progressive weight loss despite normal appetite and occasional lethargy. The cat lived in a shelter with several other cats, was not routinely vaccinated and dewormed, and was fed a commercial dry food as well as home-made food. Prior problems included a right femoral fracture after a fall, and a persistent cough that responded well to doxycycline treatment. On clinical examination, a body weight of 3.1 kg and a poor body condition were observed, along with accumulation of dental plaque, a grade IV/VI holosystolic murmur over the heart apex, and a small right kidney on abdominal palpation. Complete blood count revealed neutrophilic leukocytosis, while routine biochemical parameters, including plasma potassium and creatinine concentrations, were within normal limits (Table 1). Urinalysis revealed an appropriate specific gravity and sediment; however, the urinary protein-to-creatinine ratio was 2.3 (reference range: <0.3). Urine bacterial culture was negative.

On thoracic and abdominal radiographs, the only abnormal finding was a mild interstitial pattern in the caudal lung fields. Left ventricular free wall and interventricular septum hypertrophy was documented on echocardiography, without left atrial enlargement or other abnormalities. Abdominal ultrasonography showed a small right kidney with increased echogenicity and normal-sized and normo-echoic adrenal glands (Table 1). Arterial blood pressure was measured on the coccygeal artery using a Doppler device and appropriate cuff, in a stress-free environment, and was found to be 280-300 mmHg on repeated measurements (Brown et al., 2007). Based on this finding, a funduscopic examination was performed that revealed partial retinal detachment in the left eye. A tentative diagnosis of hypertensive hypertrophic cardiomyopathy, hypertensive retinopathy and chronic kidney disease, International Renal Interest Society (IRIS) stage I, were made. Additional test results included normal plasma total thyroxine concentration and an increased plasma aldosterone concentration (PAC) of 651 pmol/L (reference range: 195-390 pmol/L). The cat was treated with oral amlodipine (Norvasc®, Pfizer Animal Health, New York, NY, USA) at a dose of 0.625 mg q24h, and benazepril (Fortekor®, Novartis Tiergesundheit AG, Basel, Switzerland) at a dose of 0.5 mg/kg q24h, as well as on a palatable, low-sodium diet (k/d™ diet, Hill's Pet Nutrition Inc., Topeka, KS, USA). Successive measurements of systolic blood pressure revealed persistent hypertension (170-250 mmHg). In order to investigate the presence of primary hyperaldosteronism, antihypertensive treatment was withheld for two days before PAC and plasma renin activity (PRA) were measured. PAC was increased (879 pmol/L)

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Parameter	Result	Reference range
Complete blood count		
Hematocrit (%)	26.5	24-45
Hemoglobin (g/dL)	9.6	8.1-15
White blood cell count (/µL)	24350	5500-19600
Platelet count (/µL)	353000	300000-500000
Neutrophils (/µL)	22250 (91.4%)	3000-13400
Lymphocytes (/µL)	1250 (5.1%)	2000-7200
Monocytes (/µL)	240 (1%)	0-1000
Eosinophils (/µL)	610 (2.5%)	300-1700
Serum biochemistry		
Total protein (g/dL)	8.4	6.4-8.8
Albumin (g/dL)	3.6	3-4.8
Creatinine (mg/dL)	1.0	0.7-1.6
Blood urea nitrogen (mg/dL)	18	9-32
Glucose (mg/dL)	124-218	66-150
Alkaline phosphatase (U/L)	43	15-125
Alanine aminotransferase (U/L)	35	21-103
g-Glutaminotransferase (U/L)	1	1-2
Phosphorus (mg/dL)	4.1	3.5-6.7
Calcium (mg/dL)	9	8.5-11.4
Potassium (mEq/dL)	4.3	3.4-5.4
Sodium (mEq/dL)	145	144-159
Urinalysis		
Specific gravity	1.036	>1.035
Urinary protein-to-creatinine ratio (pre-treatment)	2.31	<0.3
Urinary protein-to-creatinine ratio (post-treatment)	0.3	
Endocrine tests		
Total thyroxine (nmol/L)	21	19-65
Plasma aldosterone concentration (pre-medication) (pmol/L)	651	195-390
Plasma aldosterone concentration (post-medication withdrawal) (pmol/L)	879	195-390
Plasma renin activity (post-medication withdrawal) (ng/mL/hr)	1.85	0.28-2.96
Basal urinary aldosterone-to-creatinine ratio	$92.6x10^{-9}$	$<46.5x10^{-9}$
Post-fludrocortisone urinary aldosterone-to-creatinine ratio	$155.8x10^{-9}$	$<6x10^{-9}$
Ultrasonographic findings		
Right kidney dimensions (cm)	2.7	3-4.3
Left kidney dimensions (cm)	3.3	3-4.3
Right adrenal gland dimensions (mm)	7.1 x 4.5	5-13 x 3-4.6
Left adrenal gland dimensions (mm)	8 x 4.2	5-13 x 3-4.6
Abnormal echocardiographic findings		
Left ventricular free wall (diastole) (mm)	7.77	3.1-5.9
Interventricular septum (diastole) (mm)	6.69	3.4-5.9
Left atrium / aorta	1.2	0.7-1.2

Table 1
Clinical, biochemical and ultrasonographic findings in a cat suspected of primary hyperaldosteronism, in which urinary aldosterone-to-creatinine ratio before and after fludrocortisone suppression was measured.

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and PRA was within the reference range (1.85 ng/mL/h, reference range: 0.28-2.96 ng/mL/h), resulting in an increased plasma ARR. Urinalysis revealed a normalization of the urinary protein-to-creatinine ratio. At the same time, and in order to establish an etiological diagnosis, the urinary aldosterone-to-creatinine ratio (UACR) was determined, before and after oral administration of fludrocortisone acetate, as described previously (Djajadiningrat-Laanen et al., 2008). The cat was hospitalized, and on the first day a urine sample was obtained by cystocentesis before the daily administration of fludrocortisone acetate (Florinef®, Bristol-Myers Squibb, Princeton, NJ, USA) at a dose of 0.05 mg/kg q12h for four consecutive days. Intermittent blood pressure monitoring documented a persistent, but moderate systemic arterial hypertension (<220 mmHg); however, the cat was alert and stable during the test period, and funduscopic examination during that time did not reveal any changes. On the fifth day, a second morning urine sample was obtained and the UACR was measured in both samples. The basal UACR was $92.6x10^{-9}$ (reference range: $<46.5x10^{-9}$) and the UACR after fludrocortisone administration was $155.8x10^{-9}$ (reference range: $<6x10^{-9}$) (Djajadiningrat-Laanen et al., 2008). The increased basal ratio and the complete lack of suppression that is otherwise expected from normal adrenal glands strongly supported the hypothesis of primary hyperaldosteronism. Antihypertensive therapy was reinstituted; however, while waiting for the results of the suppression test, the cat died suddenly at home. Necropsy was declined by the owner.

Discussion

Primary hyperaldosteronism is typically regarded a rare disorder, but should probably rather be considered an underdiagnosed endocrinopathy in cats. Primary hyperaldosteronism is mainly characterized by systemic arterial hypertension and/or hypokalemia (Djajadiningrat-Laanen et al., 2011). It is caused by uni- or bilateral neoplasia or hyperplasia of zona glomerulosa tissue, resulting in increased aldosterone production and release into the circulation, which is the main pathophysiological mechanism behind systemic hypertension and potassium wasting (Schulman, 2010). However, as in our case, hypokalemia may not be a consistent finding (Schulman, 2010; Javadi et al., 2005). Also, arterial hypertension is not present in all cats with primary hyperaldosteronism (Schulman, 2010; Javadi et al., 2005). In addition, systemic arterial pressure is not measured on a regular basis by most veterinarians, and arterial hypertension may therefore be overlooked (Djajadiningrat-Laanen et al., 2011). Primary hyperaldosteronism may both mimic and induce chronic kidney disease (CKD) (Javadi et al., 2005), creating a conundrum as to whether CKD precedes arterial hypertension or is the result of systemic hypertension and primary hyperaldosteronism (Djajadiningrat-Laanen et al., 2011).

Although increased PAC can be indicative of primary hyperaldosteronism, it may also result from excessive activation of the renin-angiotensin system, as in secondary hyperaldosteronism. Conversely, concurrent hypokalemia should decrease PAC (Schulman, 2010; Javadi et al., 2005). PAC should therefore always be interpreted in the light of PRA and plasma potassium concentration. Suppressed PRA, by itself, is not always diagnostic for primary hyperaldosteronism since renin can also be normal or low in cats with CKD and secondary hypertension (Jensen et al., 1997; Syme et al., 2002). On the other hand, in human and feline patients with primary hyperaldosteronism and/or CKD with secondary hypertension, renin has been found to be normal or increased (Javadi et al., 2005; Flood et al., 1999; Oelkers et al., 2000; Catena et al., 2007). For these reasons, stand-alone measurement of PAC or PRA may raise the index of suspicion but cannot offer a definitive diagnosis. Laboratory sensitivity is another factor that may affect PAC and PRA measurements (Pizzolo et al., 2006; Schirpenbach et al., 2006; Stowasser, 2009).

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Measurement of the aldosterone concentration in urine collected during 24 hours has been used to assess mineralocorticoid status in humans (Cartledge and Lawson, 2000). Since this method is almost inapplicable in veterinary patients, the single-sample UACR has been suggested instead (Syme et al., 2007). However, the UACR did not differ significantly between normal, normotensive and hypertensive azotemic cats, probably because of the very low aldosterone levels in the feline urine. Even though this test was not applied to primary hyperaldosteronism cases and the results of the study of Syme et al. were not compared to plasma aldosterone concentrations, the stand-alone measurement of urinary aldosterone concentration apparently lacks sensitivity. This assumption is further supported by finding a basal UACR within the reference range in a cat with confirmed primary hyperaldosteronism (Djajadiningrat-Laanen et al., 2008). In our cat, however, the UACR did exceed the reference range, suggesting primary hyperaldosteronism.

In human patients with primary hyperaldosteronism, the fludrocortisone suppression test is used as a confirmatory test, even though the need to go beyond the ARR in order to diagnose the disease has been challenged (Gomez-Sanchez et al., 2010). In the fludrocortisone suppression test aldosterone is measured in plasma before and after four days of fludrocortisone administration. The use of the fludrocortisone suppression test by measuring UACR is a novel approach to reach a diagnosis of primary hyperaldosteronism non-invasively in cats (Djajadiningrat-Laanen et al., 2008). In contrast to oral salt loading, oral fludrocortisone acetate at a dosage of 0.05 mg/kg q12h resulted in significant aldosterone suppression in 15 healthy cats (Djajadiningrat-Laanen et al., 2008). In the same study, one cat with primary hyperaldosteronism had increased UACR after suppression, similar to our case. Even though larger feline populations are required to validate this method as a confirmatory test for primary hyperaldosteronism, the fact that it bypasses the measurement of renin activity, which can be affected by the laboratory method applied, concurrent medications, sodium status and renal function, lowers the cost and increases the reliability of the method. Its diagnostic sensitivity could theoretically be limited by the minimal excretion of aldosterone in the feline urine, as compared to humans (Syme et al., 2007).

Even though the discontinuation of antihypertensive drugs for the duration of the suppression test and the possibility of fludrocortisone-induced side effects are a concern when performing the fludrocortisone suppression test in humans (Gomez-Sanchez et al., 2010; Mulatero et al., 2010), the clinical condition of our cat remained stable throughout the test period. Since both dihydropyridines and angiotensin-converting enzyme (ACE) inhibitors can lower PAC, they may influence the results of this dynamic test (Mulatero et al., 2002). However, there are no data regarding the effect of these drugs on aldosterone secretion in cats with primary hyperaldosteronism. In our case the washout period, two days, was suboptimal, since, in humans, discontinuation of dihydropyridines and ACE inhibitors is suggested to be of at least two weeks' duration (Funder et al., 2008). This seems to suggest that their effect was present throughout the duration of the fludrocortisone suppression test, but UACR values were increased nevertheless. Therefore, altering the medication status, especially in the setting of hypertensive target-organ pathology, is not warranted until substantiated by potential future studies.

A definitive diagnosis of primary hyperaldosteronism was not possible in our case, since a post-mortem examination, that could possibly have verified a microadenoma or hyperplasia of the zona glomerulosa, was not performed. Abdominal ultrasonography,

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which was unremarkable in our cat, in conjunction with computed tomography or magnetic resonance imaging, has been used in previous cases to assess the adrenal glands for unilateral or bilateral masses (Ash et al., 2005). However, diagnostic imaging techniques are not always accurate in establishing the etiology of primary hyperaldosteronism in cats (Djajadiningrat-Laanen et al., 2011).

Proteinuria and systemic hypertension on admission are compatible with an underlying IRIS stage I CKD with arterial hypertension in the cat of our report. It is unknown whether CKD was the result of primary hyperaldosteronism, or whether the severe hypertension can be explained solely by the concurrent IRIS stage I renal failure. However, the high PAC on admission and the results of the fludrocortisone suppression test are highly suggestive of primary hyperaldosteronism.

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Summarizing discussion and conclusions

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Primary hyperaldosteronism or low-renin hyperaldosteronism in cats is characterized by inappropriately increased aldosterone secretion from either unilateral or bilateral neoplasia or bilateral nodular hyperplasia of the adrenal zona glomerulosa (Galac et al., 2010). The resulting mineralocorticoid excess results in increased sodium and water retention and increased potassium excretion in the kidneys, with systemic arterial hypertension and hypokalemia as leading manifestations. Depending on the etiology, primary hyperaldosteronism can be treated surgically or pharmacologically, by which the hypertension and hypokalemia may be cured or alleviated. The investigation of cats with arterial hypertension and/or hypokalemia for possible abnormal aldosterone regulation is therefore highly relevant.

Chronic kidney disease is relatively common in cats (Polzin, 2010) and has been associated with systemic arterial hypertension and hypokalemia, which are also hallmarks of primary hyperaldosteronism. It is assumed that the renin-angiotensin-aldosterone system plays a role in the pathogenesis of arterial hypertension and hypokalemia in cats with chronic renal disease, but the mechanism has not been elucidated. Variable activation of the renin-angiotensin-aldosterone axis was found in 11 cats with chronic kidney disease and concurrent systemic arterial hypertension (Jensen et al., 1997), but treatment with an inhibitor of angiotensin-converting enzyme (ACE) did not effectively lower systolic blood pressure (Jensen et al., 1997), nor did it significantly increase survival time in cats with chronic kidney disease (King et al., 2006; Mizutani et al., 2006). The pathophysiology of arterial hypertension, hypokalemia and chronic kidney disease may therefore involve other pathways than ACE-induced angiotensin II production.

In addition to its classical mineralocorticoid effects, aldosterone also has pro-inflammatory and profibrotic properties (Rocha et al., 1999; Sun et al., 2002), and primary hyperaldosteronism may therefore bring about vasculopathy. Thrombotic and vascular proliferative lesions in the kidneys have been demonstrated in response to exogenous aldosterone administration in rodent models (Rocha et al., 1999; Blasi, 2003). There is increasing evidence that these non-epithelial actions of aldosterone can promote and accelerate progressive kidney disease in humans (Farquharson and Struthers, 2002; Hollenberg, 2004) and it has been hypothesized that this may also occur in cats. **Chapter 3** describes 11 cats with low-renin hyperaldosteronism. One of these cats developed chronic kidney disease during the study period and in several others there was demonstrable progression of chronic kidney disease. Histological examination of the kidneys of two of these cats revealed severe chronic inflammatory changes in the glomeruli, interstitium and arteries. In contrast with all previously reported cases of feline primary hyperaldosteronism, bilateral nodular hyperplasia of the adrenal zona glomerulosa was confirmed histologically in three of these cats and suspected in the other eight. Plasma aldosterone concentrations (PAC) in these cats ranged from within the reference range to greatly elevated levels. It was hypothesized that the relatively mild hyperaldosteronism in these cats with presumed or confirmed hyperplastic zona glomerulosa tissue was not sufficient to completely suppress plasma renin activity (PRA), and that the resulting exposure to elevated plasma levels of both aldosterone and angiotensin II caused vascular changes and fibroproliferative destruction of the kidneys. Although the findings presented in Chapter 3 suggest that primary hyperaldosteronism may play a role in the development and/or progression of renal failure in cats, further studies are required to determine whether primary hyperaldosteronism is independently associated with initiation or progression of chronic kidney disease in this species.

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The association between progressive kidney disease and low-renin hyperaldosteronism in the cats described in Chapter 3 prompted exploratory investigation of the prevalence of primary hyperaldosteronism in cats with chronic kidney disease, as described in **Chapter 4**. Chronic kidney disease was defined by the occurrence of plasma creatinine concentrations repeatedly exceeding 140 µmol/L, without concurrent clinical signs suggestive of an extrarenal cause for the elevation. Fifty-one cats with chronic kidney disease were examined for primary hyperaldosteronism using the plasma aldosterone-to-renin ratio as a screening test. Seven of the cats (14%) had an elevated plasma aldosterone-to-renin ratio, pointing to inappropriately high aldosterone secretion. Although the suspicion of primary hyperaldosteronism should ideally have been confirmed using other diagnostic means, the preliminary finding of inappropriate aldosterone secretion in 14% of the examined cats suggests that investigation for primary hyperaldosteronism is warranted in cats with chronic kidney disease, especially since treatment of primary hyperaldosteronism, either surgical or with aldosterone receptor blockers, and potentially angiotensin-II receptor blockers in case of bilateral nodular hyperplasia, could potentially delay progression of kidney disease.

Although hypokalemia, arterial hypertension and/or chronic kidney disease are all reasons to consider the diagnosis of hyperaldosteronism in cats, the investigation can be rather complicated and detection rates have historically been quite low. A limited awareness of the disease, a premature conclusion that chronic renal disease is the cause of hypokalemia and/or arterial hypertension rather than a consequence of primary hyperaldosteronism, and an inability to routinely measure PAC and PRA may all contribute to the underdiagnosis of primary hyperaldosteronism in cats.

The ratio between PAC and PRA, termed the aldosterone-to-renin ratio (ARR), has been widely accepted as a screening test for primary hyperaldosteronism in cats (Javadi et al., 2004; Javadi et al., 2005; Briscoe et al., 2009; Willi et al., 2012). The ARR is more appropriate than the PAC alone because the latter can also rise as a physiological response to increased renin levels, as in secondary hyperaldosteronism. Moreover, the simultaneous evaluation with PRA will reveal whether a within-reference range PAC is inappropriately high. For example, two cats with histopathologically confirmed bilateral nodular hyperplasia of the zona glomerulosa, described in Chapter 3, had within-reference range PAC. However, PRA was low, and consequently the ARR was elevated, disclosing inappropriate aldosterone secretion. Requiring an elevated PAC in addition to an elevated ARR for a positive screening test would have excluded these cats from diagnosis. The ARR is clearly a better screening test for primary hyperaldosteronism in cats than is the PAC alone or is the combined evaluation of ARR and PAC (Javadi et al., 2005).

In spite of this, the ARR has some practical limitations. A large (4 mL) blood sample is required for measurement of PAC and PRA. To preserve the enzymatic activity of renin the sample must be cooled immediately and the correct collection, processing and shipping are rather laborious. Reference values vary considerably between laboratories, making comparison difficult. Furthermore, renin is not the only regulator of aldosterone secretion and therefore false-positive and false-negative test results are possible. Finally, the ratio only reveals the aldosterone and renin levels at a specific point in time, and due to fluctuations in secretion of both, a single value within the reference range does not exclude primary hyperaldosteronism in cats (Javadi et al., 2005).

To circumvent these limitations of the ARR in plasma, measuring aldosterone in urine was explored, as described in **Chapter 5**. Urine is easily obtained from most cats and using the urinary aldosterone concentration avoids the above problems with plasma renin.

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In addition, urinary aldosterone excretion reflects aldosterone secretion over a longer period of time than does a single measurement of aldosterone in plasma, thereby potentially reducing false-negative and false-positive test results.

Basal urinary aldosterone excretion was measured in 42 healthy cats and one cat with a confirmed aldosterone-producing adrenocortical carcinoma. The basal urinary aldosterone-to-creatinine ratio (UACR) in the cat with primary hyperaldosteronism was within the reference range of $<46.5 \times 10^{-9}$ in the healthy cats. This indicated the need for a suppression test to reveal autonomous hypersecretion of aldosterone.

Such a test based on suppression of the UACR by either sodium chloride or fludrocortisone acetate was explored (Chapter 5). Sodium chloride was administered in the food of 22 healthy cats in a dose of 0.25 g/kg body weight q12h on four consecutive days. All cats ingested the full dose of sodium chloride, but the sodium-to-creatinine ratio in morning urine samples failed to increase by at least 100% in 12 of them and in the other 10 cats it did increase but failed to cause a significant decrease in the UACR. Consequently, the oral sodium loading test was not deemed to be useful in cats.

In contrast, the administration of fludrocortisone acetate in a dose of 0.05 mg/kg body weight q12h for four consecutive days caused a large, significant decrease in UACR in all 15 healthy cats to which it was administered. The median basal UACR decreased from 6.9×10^{-9} to 2.2×10^{-9}, a median suppression of 78% (range 44-97%). In contrast, the UACR remained high in the cat with confirmed primary hyperaldosteronism, suggesting that this test might prove to be useful and deserves further evaluation.

The study reported in **Chapter 6** was designed to evaluate the efficacy, as well as the safety, of the oral fludrocortisone suppression test to confirm or exclude the diagnosis of primary hyperaldosteronism in cats with hypokalemia or arterial hypertension, or both. The test was performed in 19 cats with hypokalemia and/or arterial hypertension related to primary hyperaldosteronism (PHA group, nine cats) or other causes (non-PHA group, ten cats). Changes in urinary aldosterone excretion were monitored from day to day to determine the minimum duration of the test. Side effects, such as a transient decrease in the plasma potassium concentration or a rise in arterial blood pressure, were also documented.

Basal UACRs in cats with primary hyperaldosteronism overlapped with those in cats with arterial hypertension or hypokalemia and arterial hypertension due to other causes, but basal UACRs above the reference range were only found in cats with primary hyperaldosteronism, and in all cats with primary hyperaldosteronism the basal UACR was $>7.5 \times 10^{-9}$. In other words, an elevated basal UACR indicated primary hyperaldosteronism and a basal UACR $<7.5 \times 10^{-9}$ excluded it. In cats with UACRs between 7.5×10^{-9} and the upper limit of the reference range, the fludrocortisone suppression test was required for differentiation. As in healthy cats, four days of oral fludrocortisone administration induced >50% suppression in all non-PHA cats with basal UACRs $>7.5 \times 10^{-9}$. In contrast, fludrocortisone administration resulted in <50% suppression in six of the nine PHA cats. Applying three criteria – (1) a basal UACR $<7.5 \times 10^{-9}$ excludes primary hyperaldosteronism; (2) a basal UACR above the reference range points to primary hyperaldosteronism; and (3) in cats with a basal UACR between 7.5×10^{-9} and 46.5×10^{-9}, <50% suppression of the UACR confirms primary hyperaldosteronism – correctly excluded primary hyperaldosteronism in all cats in which the ARR was not elevated, and correctly indicated primary hyperaldosteronism in seven of the nine cats in which the ARR was elevated. One of the two remaining cats had a histologically confirmed multinodular lesion of the zona glomerulosa, and the other had no detectable abnormalities by ultrasonographic examination of the adrenals. It was hypothesized that

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the presence of hyperplastic zona glomerulosa tissue in these cats might have left the adrenal glands partially susceptible to normal regulation of aldosterone production and secretion.

The considerable variation in both basal and suppressed UACRs in cats with primary hyperaldosteronism of all causes indicated that neither the UACR nor the degree of suppression can be used to predict the cause, i.e. adenoma, adenocarcinoma or bilateral hyperplasia of the zona glomerulosa.

Side effects of fludrocortisone administration in these 19 cats were limited to changes in plasma potassium concentration. Decreases >1 mmol/L occurred in three cats and hypokalemia was induced or exacerbated in seven, resulting in muscle weakness in one. Therefore daily measurement of plasma potassium concentration would seem advisable to optimize potassium supplementation during the test period.

Results of both the ARR and the fludrocortisone suppression test may be influenced by concurrent medication. Guidelines for ARR and fludrocortisone suppression testing in humans advise that agents that markedly affect the ARR, such as spironolactone and potassium-wasting diuretics, be discontinued for at least four weeks. Also, if the result of the ARR is inconclusive, other potentially interfering agents, such as beta-adrenergic blockers, dihydropyridine calcium channel antagonists, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers, should be discontinued for two weeks, if possible (Funder et al., 2008). Unfortunately, most cats with primary hyperaldosteronism have severe arterial hypertension, which is best controlled with a dihydropyridine calcium channel antagonist (e.g. amlodipine), either alone or in combination with a beta-adrenergic blocker or an angiotensin-converting enzyme inhibitor (Brown et al., 2007). Discontinuing these antihypertensive agents carries the risk of loss of control of arterial pressure and thus further damage to the kidneys, eyes, heart and brain. Until the effect of these medications on the ARR and fludrocortisone suppression test in cats has been documented, it is probably advisable not to discontinue them during the tests.

Taking into account the findings in this thesis, the following protocol is suggested. Primary hyperaldosteronism should be suspected in any cat presented with arterial hypertension, hypokalemia and/or chronic kidney disease. In addition to a thorough physical examination and measurement of arterial blood pressure (Brown et al., 2007), the diagnostic investigation should include urinalysis and measurement of plasma concentrations of creatinine, urea, sodium, potassium, calcium, phosphate, thyroxine, glucose and fructosamine. The detection of a low-normal plasma phosphate concentration in a cat with chronic kidney disease may raise suspicion of primary hyperaldosteronism (Chapter 3), but is not a consistent finding in cats with this disorder (Chapter 4 and Chapter 6).

The plasma aldosterone-to-renin ratio (ARR) may be used to reveal inappropriately high aldosterone secretion in these patients. The suspicion of primary hyperaldosteronism is increased if hypokalemia is found in conjunction with an elevated plasma aldosterone concentration (PAC), because hypokalemia normally suppresses aldosterone secretion. If the ARR is within the reference range, serial sampling may be required to finally reveal an elevated ARR. This may be especially relevant in cats with primary hyperaldosteronism caused by bilateral nodular hyperplasia of the zona glomerulosa, and in cats receiving medication that potentially influences the ARR.

In cats with an elevated ARR and cats whose ARR is within the reference range but are nevertheless strongly suspected of having primary hyperaldosteronism, an additional test should be used to confirm or exclude primary hyperaldosteronism. Extrapolating from suppression testing in humans, the cat should probably be

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normotensive and potassium-replete before commencing the oral fludrocortisone suppression test, and medications that markedly affect the ARR, such as spironolactone, should be discontinued for at least four weeks. It may be necessary to monitor the plasma potassium concentration during the test in order to optimize oral potassium supplementation. Morning samples are collected for determination of the urinary aldosterone-to-creatinine ratio (UACR), before and after suppression with fludrocortisone acetate in a dose of 0.05 mg/kg body weight q12h for four days. A basal UACR $<7.5x10^{-9}$ excludes primary hyperaldosteronism and a value $>46.5x10^{-9}$ confirms it, while for values between $7.5x10^{-9}$ and $46.5x10^{-9}$, suppression by <50% also confirms it.

In cats with confirmed primary hyperaldosteronism, diagnostic imaging is employed to determine the cause and to detect distant metastases in cases of adrenocortical neoplasia. If diagnostic imaging does not reveal an adrenocortical abnormality, the possibility of false-negative diagnostic imaging results should be considered.

For confirmed, non-metastasized, unilateral adrenocortical neoplasia, surgical treatment is preferable, although associated with a considerable perioperative risk of intra-abdominal hemorrhage. Medical therapy with a mineralocorticoid receptor blocker such as spironolactone, with potassium supplementation and antihypertensive drugs if needed, is instituted in cats with bilateral hyperplasia or a non-resectable neoplasm of the zona glomerulosa, or other factors precluding surgery. The additional use of an angiotensin-II type 1 (AT_1) receptor blocker such as telmisartan, which has recently been introduced in veterinary medicine, needs to be investigated but might prove beneficial in cats with bilateral nodular hyperplasia of the adrenal zona glomerulosa, which may be subjected to the combined deleterious effects of elevated plasma aldosterone and angiotensin II levels.

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Conclusions

Primary hyperaldosteronism in cats may be caused by bilateral nodular hyperplasia of the zona glomerulosa, as well as by adrenocortical neoplasia.

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Primary hyperaldosteronism may play a role in the development and/or progression of chronic kidney disease in cats, but further studies are needed to determine whether primary hyperaldosteronism is independently associated with initiation or progression of chronic kidney disease in this species.

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Investigation for hyperaldosteronism is warranted in cats with chronic kidney disease, especially since successful treatment of hyperaldosteronism might delay progression of the kidney disease.

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The ratio of plasma aldosterone concentration (PAC) to plasma renin activity (PRA), called the aldosterone-to-renin ratio (ARR), is a better screening test for primary hyperaldosteronism in cats than is the PAC alone.

–

In some cats with primary hyperaldosteronism, especially in those with bilateral nodular hyperplasia of the zona glomerulosa, PAC is not elevated. Requiring an elevated PAC in addition to an elevated ARR for a positive screening test will exclude these cats from diagnosis.

–

In cats with primary hyperaldosteronism, the basal urinary aldosterone-to-creatinine ratio (UACR) can be within the reference range, which necessitates a suppression test to confirm the autonomous hypersecretion of aldosterone.

–

A confirmative test for primary hyperaldosteronism based on suppression of the UACR by sodium chloride is not useful in cats, but the suppression test with fludrocortisone is very useful.

–

Neither an elevated basal UACR nor a specific level of suppression by fludrocortisone acetate can be used to predict the cause of primary hyperaldosteronism, i.e. adenoma, adenocarcinoma or bilateral hyperplasia of the zona glomerulosa.

–

In cats with suspected primary hyperaldosteronism the fludrocortisone suppression test based on the UACR can be interpreted as follows:

1. basal UACR $<7.5 \times 10^{-9}$ excludes primary hyperaldosteronism;
2. basal UACR $>46.5 \times 10^{-9}$ confirms primary hyperaldosteronism;
3. for basal UACR between 7.5×10^{-9} and 46.5×10^{-9}, suppression of UACR <50% confirms primary hyperaldosteronism.

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Samenvattende discussie en conclusies

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Primair hyperaldosteronisme, ook aangeduid als het syndroom van Conn, wordt bij katten gekenmerkt door een autonome productie van aldosteron door één of beide bijnieren, als gevolg van ofwel een eenzijdige of beiderzijdse neoplasie, ofwel beiderzijdse hyperplasie van de zona glomerulosa (Galac et al., 2010). De overmatige secretie van mineralocorticoïden leidt in de nieren tot sterkere natrium- en waterretentie en verhoogde kaliumuitscheiding, met systemische arteriële hypertensie en hypokalemie als meest opvallende gevolgen. Primair hyperaldosteronisme kan, afhankelijk van de oorzaak, chirurgisch of medicamenteus worden behandeld, met als belangrijkste doelstelling het normaliseren van de arteriële bloeddruk en plasma-kaliumconcentratie. Het is dus relevant om bij katten met arteriële hypertensie en/of hypokalemie te onderzoeken of er sprake is van een stoornis in de regulatie van de aldosteronproductie.

Chronisch nierlijden komt relatief veel voor bij katten (Polzin, 2010) en kan gepaard gaan met arteriële hypertensie en hypokalemie, twee problemen die ook bij uitstek worden gezien bij katten met primair hyperaldosteronisme. Er wordt aangenomen dat het renine-angiotensine-aldosteron-systeem een rol speelt in de pathogenese van arteriële hypertensie en hypokalemie bij katten met chronisch nierlijden, maar het mechanisme is nog niet opgehelderd. Hoewel bij elf katten met zowel chronisch nierlijden als arteriële hypertensie variabele activatie van het renine-angiotensine-aldosteron-systeem werd beschreven (Jensen et al., 1997), leidde behandeling van katten met chronisch nierlijden met een 'angiotensin-converting enzyme' (ACE)-remmer niet tot een significante verlaging van de bloeddruk (Jensen et al., 1997) of een significante toename van de overlevingsduur (King et al., 2006; Mizutani et al., 2006). Bij de pathofysiologie van arteriële hypertensie, hypokalemie en chronisch nierlijden zouden dus andere factoren dan door ACE geïnduceerde angiotensine II-productie een rol kunnen spelen.

Naast klassieke mineralocorticoïde effecten heeft aldosteron ook niet-epitheliale, proinflammatoire effecten (Rocha et al., 1999; Sun et al., 2002). Blootstelling aan hoge plasmaconcentraties van aldosteron kan dan ook leiden tot het ontstaan van een vasculopathie. Zo veroorzaakte aldosterontoediening in een knaagdiermodel trombose en proliferatie van bloedvaten in de nieren (Rocha et al., 1999; Blasi, 2003). Steeds meer onderzoeksbevindingen duiden erop dat de proinflammatoire effecten van aldosteron het ontstaan en de progressie van nierlijden kunnen bevorderen bij mensen (Farquharson and Struthers, 2002; Hollenberg, 2004), en er wordt verondersteld dat dit ook het geval kan zijn bij katten. In **hoofdstuk 3** worden elf katten met primair hyperaldosteronisme beschreven. Eén van deze katten ontwikkelde tijdens het onderzoek chronisch nierlijden en bij diverse andere katten trad progressie van bestaand chronisch nierlijden op. Van twee katten werden de nieren histopathologisch onderzocht. Hierbij werden ernstige, chronische ontstekingsverschijnselen in de glomeruli, het interstitium en de arteriën gezien. Bij drie katten werd nodulaire hyperplasie van de zona glomerulosa van de bijnieren aangetoond door middel van histopathologisch onderzoek en bij de andere acht katten werd beiderzijdse nodulaire hyperplasie vermoed op basis van de klinische en klinisch-chemische bevindingen en het ontbreken van afwijkingen bij diagnostische beeldvorming van de bijnieren. De bewezen of vermoedelijke hyperplasie van de zona glomerulosa bij deze elf katten vormde een opvallende tegenstelling met alle tot dan toe beschreven katten met primair hyperaldosteronisme, waarbij steeds sprake was geweest van een neoplastische etiologie. Bij de katten in hoofdstuk 3 varieerde de plasma-aldosteronconcentratie (PAC) van binnen het referentiegebied tot sterk verhoogde waarden. Er werd verondersteld dat de PAC bij deze katten niet hoog genoeg was om de plasma-renine-activiteit (PRA) – en daarmee de productie van angiotensine II –

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volledig te remmen, en dat de resulterende blootstelling aan verhoogde concentraties van zowel PAC als angiotensine II de oorzaak was van vaatveranderingen en fibroproliferatieve destructie van de nieren. Hoewel de bevindingen in hoofdstuk 3 het aannemelijk maken dat primair hyperaldosteronisme een rol speelt bij de ontwikkeling en/of progressie van chronisch nierlijden bij katten, is verder onderzoek nodig om te bepalen of er een onafhankelijk causaal verband bestaat tussen primair hyperaldosteronisme en het ontstaan of de progressie van nierlijden bij deze diersoort.

Het gelijktijdig voorkomen van progressief nierlijden en primair hyperaldosteronisme bij de in hoofdstuk 3 beschreven katten was de aanleiding voor het verkennende onderzoek naar de prevalentie van primair hyperaldosteronisme bij katten met chronisch nierlijden, zoals beschreven in **hoofdstuk 4**. Chronisch nierlijden werd gedefinieerd als een bij herhaling verhoogde plasma-kreatinineconcentratie (>140 µmol/l) die niet gepaard gaat met verschijnselen die zouden kunnen duiden op een extrarenale oorzaak van de verhoogde plasma kreatinineconcentratie. Eénenvijftig katten met chronisch nierlijden werden onderzocht op aanwijzingen voor primair hyperaldosteronisme door bepaling van de aldosteron-renine ratio in het bloedplasma. Bij zeven katten (14%) werd een verhoogde aldosteron-renine ratio, suggestief voor autonome aldosteronsecretie, gevonden. Idealiter zou primair hyperaldosteronisme bevestigd zijn met behulp van andere onderzoeksmethoden. Dat is in dit onderzoek niet gebeurd. Toch lijkt het, op basis van de aanwijzingen voor autonome aldosteronsecretie bij 14% van de onderzochte katten, gerechtvaardigd om katten met chronisch nierlijden routinematig te onderzoeken op primair hyperaldosteronisme, eens te meer omdat chirurgische of medicamenteuze behandeling van primair hyperaldosteronisme de progressie van het nierlijden mogelijk zou kunnen vertragen.

Hoewel primair hyperaldosteronisme deel uitmaakt van de differentiële diagnose van hypokalemie, arteriële hypertensie en/of chronisch nierlijden bij de kat, kan het vrij lastig zijn om deze aandoening te diagnosticeren. De diagnose wordt dan ook weinig gesteld. Verschillende factoren zijn debet aan het onderdiagnosticeren van primair hyperaldosteronisme bij katten, waaronder de beperkte bekendheid van de ziekte, het niet routinematig kunnen meten van PAC en PRA en, bij katten met zowel chronisch nierlijden als hypokalemie en/of arteriële hypertensie, de voorbarige conclusie dat het chronisch nierlijden de oorzaak van de hypokalemie en/of arteriële hypertensie is.

De verhouding tussen PAC en PRA, ook wel de aldosteron-renine ratio (ARR) genoemd, wordt algemeen gezien als een goede ‘screeningstest’ voor primair hyperaldosteronisme bij katten (Javadi et al., 2004; Javadi et al., 2005; Briscoe et al., 2009; Willi et al., 2012). De ARR is een betere screeningstest voor primair hyperaldosteronisme bij katten dan de PAC (Javadi et al., 2005), omdat de PAC ook kan stijgen als fysiologische reactie op verhoogde renineafgifte, zoals bij secundair hyperaldosteronisme. Daarnaast zal de gelijktijdige bepaling van de PRA uitwijzen of een PAC, die op zich binnen de referentiewaarden valt, wel in verhouding staat tot de PRA; met andere woorden, of de PAC niet hoger is dan verwacht op basis van de plasma-renine-activiteit. Tot voorbeeld dienen twee katten uit hoofdstuk 3, met histopathologisch bevestigde, beiderzijdse nodulaire hyperplasie van de zona glomerulosa. De PAC van deze katten viel binnen de referentiewaarden, maar de PRA was opmerkelijk laag. De verhoogde ARR onthulde dat de aldosteronsecretie, tenminste gedeeltelijk, autonoom geschiedde. Als de combinatie van een verhoogde ARR én een verhoogde PAC zou zijn gebruikt als diagnostisch criterium, was de diagnose primair hyperaldosteronisme bij deze katten gemist.

Naast voordelen kent de ARR ook enkele praktische beperkingen. Voor het meten

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van de PAC en de PRA is een groot (4 ml) bloedmonster nodig. Het monster moet direct na afname worden gekoeld om verlies van de enzymactiviteit van renine te beperken. Het is ook vrij bewerkelijk om het bloedmonster op de juiste manier af te nemen, te verwerken en te verzenden. Bovendien lopen referentiewaarden sterk uiteen tussen de verschillende laboratoria, waardoor onderlinge vergelijking moeilijk is. Verder is renine niet de enige regulator van de aldosteronsecretie en daardoor zijn fout-positieve en fout-negatieve testresultaten mogelijk. Tot slot geeft de ratio alleen de hoogte van aldosteron en renine op een bepaald tijdstip weer. Als gevolg van fluctuaties in de secretie van aldosteron en renine bij een kat kan primair hyperaldosteronisme niet worden uitgesloten op basis van één enkele ARR die binnen de referentiewaarden valt (Javadi et al., 2005).

Om deze beperkingen van de ARR te omzeilen werd de bepaling van de aldosteronexcretie in de urine onderzocht, zoals beschreven in **hoofdstuk 5**. Urine kan bij de meeste katten gemakkelijk worden verkregen. Door de aldosteronconcentratie in urine te bepalen worden de eerder genoemde problemen met de plasma-renine-activiteit uit de weg gegaan. In tegenstelling tot de PAC, die de aldosteronsecretie op één moment weergeeft, is de aldosteronconcentratie in de ochtendurine een afspiegeling van de aldosteronsecretie over een langere tijdsperiode, waarmee de kans op fout-negatieve en fout-positieve testresultaten mogelijk wordt gereduceerd.

De basale aldosteron-kreatinine ratio in de ochtendurine (urinary aldosterone-to-creatinine ratio, UACR) werd gemeten bij 42 gezonde katten en op basis van deze waarden werd een referentiegebied berekend van $<46{,}5\times10^{-9}$. De basale UACR werd ook bepaald bij één kat met primair hyperaldosteronisme ten gevolge van een aldosteronproducerend bijniercarcinoom. De basale UACR bleek bij deze kat in het referentiegebied te liggen. Dit wees erop dat een suppressietest nodig zou zijn om het autonome karakter van de hypersecretie van aldosteron aan te tonen.

Een dergelijke test, gebaseerd op de remming van de aldosteronproductie door natriumchloride of fludrocortisonacetaat, werd onderzocht in hoofdstuk 5. Natriumchloride werd gemengd door het voer van 22 gezonde katten, in een dosering van 2x daags 0,25 g/kg lichaamsgewicht op vier achtereenvolgende dagen. Hoewel alle katten de volledige dosering natriumchloride innamen, steeg de natrium-kreatinine ratio in de ochtendurine bij twaalf katten slechts met minder dan 100%, zodat de orale natriumbelasting als niet geslaagd werd beschouwd. Bij de andere tien katten steeg de natrium-kreatinine ratio in de ochtendurine wel met tenminste 100%, maar bij deze katten veroorzaakte de natriumbelasting geen significante remming van de UACR. Daarmee bleek orale natriumbelasting geen bruikbare suppressietest te zijn voor katten die ervan worden verdacht te lijden aan primair hyperaldosteronisme.

De toediening van fludrocortisonacetaat in een dosering van 2x daags 0,05 mg/kg lichaamsgewicht gedurende vier achtereenvolgende dagen bleek wel een sterke, significante daling van de UACR te veroorzaken bij de vijftien gezonde katten waaraan het werd toegediend. De mediane waarde van de basale UACR daalde van $6{,}9\times10^{-9}$ tot $2{,}2\times10^{-9}$, hetgeen neerkwam op een mediane suppressie van 78% (spreiding: 44-97%). De UACR bleef echter hoog bij de kat met bevestigd primair hyperaldosteronisme. Dit suggereerde dat deze test bruikbaar zou kunnen zijn als suppressietest voor katten die ervan worden verdacht te lijden aan primair hyperaldosteronisme, en dat de test het verdiende om verder te worden geëvalueerd.

In het onderzoek dat wordt beschreven in **hoofdstuk 6**, werd onderzocht of de orale fludrocortison suppressietest veilig en geschikt is om de diagnose primair hyperaldosteronisme te bevestigen of uit te sluiten bij katten met hypokalemie, arteriële

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hypertensie, of beide. De test werd uitgevoerd bij negentien katten met hypokalemie en/of arteriële hypertensie, samenhangend met primair hyperaldosteronisme (PHA-groep, negen katten) of andere oorzaken (non-PHA-groep, tien katten). De aldosteronexcretie in de urine werd dagelijks bepaald teneinde de minimaal benodigde duur van de suppressietest vast te kunnen stellen. Bijwerkingen, zoals een tijdelijke daling van de plasma-kaliumconcentratie of stijging van de arteriële bloeddruk, werden ook genoteerd.

De basale UACR bij katten met primair hyperaldosteronisme en bij katten met arteriële hypertensie en/of hypokalemie door andere oorzaken bleken te overlappen, maar bij alle katten met primair hyperaldosteronisme bedroeg de basale UACR >7,5x10^{-9} en een verhoogde basale UACR werd alleen gevonden bij katten met primair hyperaldosteronisme. Met andere woorden, de diagnose primair hyperaldosteronisme werd bevestigd bij een verhoogde basale UACR en kon worden uitgesloten bij een basale UACR <7,5x10^{-9}. Bij katten met een UACR tussen 7,5x10^{-9} en 46,5x10^{9}, de bovengrens van het referentiegebied, was de fludrocortison suppressietest noodzakelijk om onderscheid te kunnen maken tussen katten met en zonder primair hyperaldosteronisme. Orale fludrocortisontoediening gedurende vier dagen leidde bij alle katten uit de non-PHA-groep met een basale UACR >7,5x10^{-9} – net als bij gezonde katten – tot >50% remming van de aldosteronexcretie, terwijl bij zes van de negen katten uit de PHA-groep <50% remming van de aldosteronexcretie optrad.

Aan de hand van de onderzoeksbevindingen werden drie criteria opgesteld voor de interpretatie van de basale UACR en de fludrocortison suppressietest: (1) een basale UACR <7,5x10^{-9} sluit primair hyperaldosteronisme uit; (2) een verhoogde basale UACR bevestigt primair hyperaldosteronisme; en (3) bij katten met een basale UACR tussen 7,5x10^{-9} en 46,5x10^{-9} wordt primair hyperaldosteronisme bevestigd bij een remmingspercentage van <50%. Het toepassen van deze criteria op de negentien katten met hypokalemie en/of arteriële hypertensie resulteerde in het terecht uitsluiten van primair hyperaldosteronisme bij alle katten met een ARR binnen de referentiewaarden en tot het terecht bevestigen van primair hyperaldosteronisme bij zeven van de negen katten met een verhoogde ARR. Bij één van de twee overgebleven katten uit de PHA-groep werd bij histopathologisch onderzoek van de bijnier een multinodulaire laesie van de zona glomerulosa vastgesteld, terwijl echografisch onderzoek van de bijnieren bij de andere kat geen afwijkingen opleverde. Er werd verondersteld dat hyperplastisch zona glomerulosaweefsel bij deze katten nog gedeeltelijk gevoelig was voor normale regulatie van aldosteronproductie en -secretie.

De aanzienlijke variatie in zowel de basale UACR als de UACR na suppressie bij katten met primair hyperaldosteronisme van verschillende etiologie wees erop dat noch de UACR noch het remmingspercentage bruikbaar is om de oorzaak van het primaire hyperaldosteronisme – dat wil zeggen, adenoom, adenocarcinoom, of beiderzijdse hyperplasie van de zona glomerulosa – te voorspellen.

Bijwerkingen van fludrocortison bij deze negentien katten bleven beperkt tot verlaging van de plasma-kaliumconcentratie. Fludrocortisontoediening leidde bij drie katten tot een afname in de plasma-kaliumconcentratie van >1 mmol/l, en bij zeven katten tot inductie of versterking van hypokalemie, hetgeen bij één kat gepaard ging met spierzwakte. Om deze reden lijkt het aan te bevelen om de kaliumsuppletie tijdens de fludrocortison suppressietest te optimaliseren op basis van dagelijkse bepaling van de plasma-kaliumconcentratie.

De resultaten van zowel de ARR als de fludrocortison suppressietest kunnen zijn beïnvloed door medicatie. Volgens de richtlijnen voor de ARR en de fludrocortison suppressietest bij mensen dient medicatie, die de ARR sterk beïnvloedt, zoals spironolacton en kaliumsparende diuretica, te worden gestaakt gedurende tenminste

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vier weken voorafgaande aan de test. Als de ARR niet diagnostisch is, dienen zo mogelijk ook andere potentieel storende farmaca, zoals beta-adrenerge blokkers, dihydropyridine calcium channel antagonisten, ACE-remmers en angiotensine receptorblokkers, gedurende tenminste twee weken te worden gestaakt (Funder et al., 2008). Helaas hebben de meeste katten met primair hyperaldosteronisme ernstige arteriële hypertensie, die het meest effectief wordt behandeld met een dihydropyridine calcium channel antagonist (amlodipine), eventueel in combinatie met een beta-adrenerge blokker of een ACE-remmer (Brown et al., 2007). Het staken van deze bloeddrukverlagende medicatie zou gepaard gaan met een aanzienlijk risico op arteriële hypertensie en verdere bloeddrukgerelateerde schade in nieren, ogen, hart en hersenen. Waarschijnlijk kan deze medicatie dus beter niet worden gestaakt totdat nader onderzoek een negatief effect op de ARR en de uitkomst van de fludrocortison suppressietest heeft aangetoond.

Naar aanleiding van de bevindingen van dit proefschrift wordt het volgende protocol voor de diagnostiek van primair hyperaldosteronisme bij katten voorgesteld. Primair hyperaldosteronisme dient te worden opgenomen in de differentiële diagnose van iedere kat met arteriële hypertensie, hypokalemie en/of chronisch nierlijden. Het onderzoek dient, naast een grondig lichamelijk onderzoek en arteriële bloeddrukmeting (Brown et al., 2007), ook urineonderzoek en bepaling van de plasmaconcentraties van kreatinine, ureum, natrium, kalium, calcium, fosfaat, thyroxine, glucose en fructosamine te omvatten. Een laag-normale plasma-fosfaatconcentratie bij een kat met chronisch nierlijden versterkt de verdenking op primair hyperaldosteronisme (hoofdstuk 3), maar is geen consistente bevinding bij katten met primair hyperaldosteronisme (hoofdstuk 4 en hoofdstuk 6).

De plasma-aldosteron-renine ratio (ARR) kan worden gebruikt om een mogelijk autonome aldosteronsecretie aan het licht te brengen. De verdenking op primair hyperaldosteronisme wordt versterkt als de patiënt zowel een verlaagde plasma-kaliumconcentratie als een verhoogde plasma-aldosteronconcentratie (PAC) heeft, omdat hypokalemie onder normale omstandigheden leidt tot verlaging van de aldosteronsecretie. Als de ARR in eerste instantie binnen de referentiewaarden valt maar de kat toch wordt verdacht van primair hyperaldosteronisme, kan het nodig zijn de bepaling te herhalen totdat uiteindelijk een verhoogde ARR wordt gevonden. Dit kan vooral het geval zijn bij katten met beiderzijdse nodulaire hyperplasie van de zona glomerulosa, en bij katten met primair hyperaldosteronisme die medicatie krijgen toegediend die mogelijk van invloed is op de ARR.

Bij katten met een verhoogde ARR en bij katten waarvan de ARR binnen de referentiewaarden valt, maar die desondanks sterk worden verdacht van primair hyperaldosteronisme, dient aanvullend onderzoek te worden uitgevoerd om primair hyperaldosteronisme te bevestigen of uit te sluiten. Extrapolerend vanuit de suppressietesten die worden gebruikt bij mensen, is het waarschijnlijk het beste om bloeddruk en plasma-kaliumconcentratie van de kat tot binnen de referentiewaarden te brengen voordat de orale fludrocortison suppressietest wordt uitgevoerd, en om medicatie die de ARR sterk beïnvloedt, zoals spironolacton, tenminste vier weken voorafgaand aan de suppressietest te staken. Het kan noodzakelijk zijn om tijdens de test de plasma-kaliumconcentratie te controleren, zodat de kaliumsuppletie kan worden geoptimaliseerd. Voor de bepaling van de urine aldosteron-kreatinine ratio (urinary aldosterone-to-creatinine ratio, UACR) wordt ochtendurine verzameld, vóór en na toediening van fludrocortisonacetaat in een dosering van 2x daags 0,05 mg/kg lichaamsgewicht gedurende vier dagen. Primair hyperaldosteronisme kan worden uitgesloten bij katten met een basale UACR $<7{,}5 \times 10^{-9}$. Primair hyperaldosteronisme

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wordt daarentegen bevestigd bij katten met ofwel een basale UACR >46,5x10^{-9}, ofwel een basale UACR tussen de 7,5x10^{-9} en 46,5x10^{-9} en <50% remming van de aldosteronexcretie na fludrocortisontoediening.

Als bij een kat primair hyperaldosteronisme is bevestigd, is diagnostische beeldvorming geïndiceerd om de oorzaak te bepalen en, in het geval van adrenocorticale neoplasie, eventuele metastasen te detecteren. Als bij diagnostische beeldvorming van de bijnieren geen afwijkingen worden gevonden, moet rekening worden gehouden met de mogelijkheid van een fout-negatieve bevinding.

Voor een bevestigde éénzijdige, niet gemetastaseerde adrenocorticale neoplasie is adrenalectomie de meest geschikte behandeling, hoewel adrenalectomie ook een aanzienlijk perioperatief risico op een intra-abdominale bloeding met zich meebrengt. Medicamenteuze behandeling met een mineralocorticoïde receptorblokker zoals spironolacton, zo nodig aangevuld met kaliumsuppletie en bloeddrukverlagende medicatie, is geïndiceerd bij katten waarbij chirurgie niet mogelijk of zinvol is vanwege bijvoorbeeld beiderzijdse hyperplasie of een inoperabele neoplasie van de zona glomerulosa. Aanvullende behandeling met een angiotensine II type 1 (AT_1) receptorblokker, zoals het recentelijk op de veterinaire markt geïntroduceerde telmisartan, moet nog worden onderzocht maar zou zinvol kunnen zijn bij katten die, ten gevolge van beiderzijdse nodulaire hyperplasie van de zona glomerulosa, blootstaan aan verhoogde plasmaconcentraties van zowel aldosteron als angiotensine II, met alle daaraan verbonden nadelige gevolgen.

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Conclusies

Primair hyperaldosteronisme kan bij katten worden veroorzaakt door zowel beiderzijdse nodulaire hyperplasie als éénzijdige of beiderzijdse neoplasie van de zona glomerulosa.

–

Primair hyperaldosteronisme speelt mogelijk een rol bij het ontstaan en/of de progressie van chronisch nierlijden bij katten. Verder onderzoek is nodig om te bepalen of er een onafhankelijk causaal verband bestaat tussen primair hyperaldosteronisme en het ontstaan of de progressie van nierlijden bij deze diersoort.

–

Bij katten met chronisch nierlijden is onderzoek naar primair hyperaldosteronisme te rechtvaardigen, in het bijzonder omdat de progressie van het nierlijden mogelijk zou kunnen worden vertraagd bij een succesvolle behandeling van het primair hyperaldosteronisme.

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De verhouding tussen de plasma-aldosteronconcentratie (PAC) en de plasma-renine-activiteit (PRA), oftewel de aldosteron-renine ratio (ARR), is een betere screeningstest voor primair hyperaldosteronisme bij katten dan de PAC.

–

Bij sommige katten met primair hyperaldosteronisme, in het bijzonder in het geval van beiderzijdse hyperplasie van de zona glomerulosa, is de PAC niet verhoogd. Het vasthouden aan een verhoogde PAC, naast een verhoogde ARR, als criterium voor een positieve screeningstest zou ertoe leiden dat de diagnose primair hyperaldosteronisme bij deze katten zou worden gemist.

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Bij katten met primair hyperaldosteronisme kan de basale aldosteron-kreatinine ratio in de urine (UACR) binnen de referentiewaarden liggen, zodat een suppressietest nodig is om het autonome karakter van de aldosteronsecretie aan te tonen.

–

Orale zoutbelasting is geen geschikte methode om, aan de hand van remming van de UACR, primair hyperaldosteronisme bij katten te bevestigen. Daarentegen is een suppressietest op basis van orale fludrocortisontoediening zeer bruikbaar.

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Noch een verhoogde basale UACR, noch het remmingspercentage na toediening van fludrocortisonacetaat, kan worden gebruikt om de oorzaak van primair hyperaldosteronisme (adenoom, adenocarcinoom, of beiderzijdse hyperplasie van de zona glomerulosa) te voorspellen.

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Bij katten, waarbij primair hyperaldosteronisme wordt vermoed, kan het resultaat van de fludrocortisonsuppressietest op basis van de UACR als volgt worden geïnterpreteerd:

1. bij een basale UACR $<7{,}5\times10^{-9}$ is primair hyperaldosteronisme uitgesloten;
2. bij een basale UACR $>46{,}5\times10^{-9}$ is primair hyperaldosteronisme bevestigd;
3. bij een basale UACR tussen $7{,}5\times10^{-9}$ en $46{,}5\times10^{-9}$ wordt primair hyperaldosteronisme bevestigd indien na fludrocortisontoediening <50% suppressie van de UACR optreedt.

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Curriculum vitae

Sylvia Djajadiningrat-Laanen was born in Rotterdam, The Netherlands, on November 2, 1968. She attended primary school at the O.S.G. Fridtjof Nansen and secondary school (VWO) at Thorbecke V.O., both in Rotterdam. She studied veterinary medicine at the Faculty of Veterinary Medicine of Utrecht University and obtained her Doctor of Veterinary Medicine degree in 1994.

After graduation she worked in private companion animal practices in and around Delft before beginning the internship in companion animal medicine in the Department of Clinical Sciences of Companion Animals of the Faculty of Veterinary Medicine of Utrecht University in 1997. In 1998 she began the residency in veterinary ophthalmology in the same department, under the tutelage of Frans Stades and Michael Boevé. In 2002 she became a Diplomate in the specialty of veterinary ophthalmology of the Royal Netherlands Veterinary Association. She successfully completed the qualifying examination of the European College of Veterinary Ophthalmologists (ECVO) and became a Diplomate in 2003. She was appointed to the staff of the Department of Clinical Sciences of Companion Animals of the Faculty of Veterinary Medicine of Utrecht University, where she continues working at present, combining clinical veterinary ophthalmology with research and teaching. She assisted in the training of several residents in ophthalmology and has been a member of the ECVO Education and Residency Committee during 2004-2010 and from 2013 onwards.

In 2003 she began a PhD research study on keratoglobus in chickens. After losing three consecutive research flocks and seeing the fourth also endangered by the recurrent threat of the avian influenza virus, she felt compelled to change her research subject. In her clinical work as an ophthalmologist she was regularly confronted with cats with acute loss of vision or intraocular hemorrhages or both, due to systemic arterial hypertension. She became especially interested in those cats in which the arterial hypertension was a consequence of primary hyperaldosteronism, and this became the topic of her new PhD research.

Sylvia is married to Tom Djajadiningrat. Their son Alwin was born in 2003, and their daughter Ilse was born in 2006.

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