J Vet Intern Med 2010;24:1093–1097

Urinary Catecholamine and Metanephrine to Creatinine Ratios in Dogs with Hyperadrenocorticism or Pheochromocytoma, and in Healthy Dogs

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Background: Urinary catecholamines and metanephrines are used for the diagnosis of pheochromocytoma (PHEO) in dogs. Hyperadrenocorticism (HAC) is an important differential diagnosis for PHEO.

Objectives: To measure urinary catecholamines and metanephrines in dogs with HAC.

Animals: Fourteen dogs with HAC, 7 dogs with PHEO, and 10 healthy dogs.

Methods: Prospective clinical trial. Urine was collected during initial work-up in the hospital; in dogs with HAC an additional sample was taken at home 1 week after discharge. Parameters were measured using high-pressure liquid chromatography and expressed as ratios to urinary creatinine concentration.

Results: Dogs with HAC had significantly higher urinary epinephrine, norepinephrine and normetanephrine to creatinine ratios than healthy dogs. Urinary epinephrine, norepinephrine, and metanephrine to creatinine ratios did not differ between dogs with HAC and dogs with PHEO, whereas the urinary normetanephrine to creatinine ratio was significantly higher ($P = .011$) in dogs with PHEO (414, 157.0–925.0, median, range versus (117.5, 53.0–323.0). Using a cut-off ratio of 4 times the highest normetanephrine to creatinine ratio measured in controls, there was no overlap between dogs with HAC and dogs with PHEO. The variables determined in urine samples collected at home did not differ from those collected in the hospital.

Conclusion and Clinical Importance: Dogs with HAC might have increased concentrations of urinary catecholamines and normetanephrine. A high concentration of urinary normetanephrine (4 times normal), is highly suggestive of PHEO.

Key words: Adrenal gland; Catecholamines; Cushing's syndrome; Endocrinology; Normetanephrine.

Pheochromocytomas (PHEOs) are catecholamine-producing neuroendocrine tumors arising from chromaffin cells of the sympathoadrenal system. Clinical signs usually result from secretion of excessive amounts of catecholamines and include among others weakness, episodic collapse, tachypnea, panting, tachycardia, pacing, polyuria/polydipsia, hypertension-associated bleeding, vomiting, and diarrhea. Occasionally, signs are attributable to the space-occupying or invasive nature of the tumor. Hormone secretion is sporadic and unpredictable, and the clinical presentation varies greatly with signs similar to many other clinical conditions.[1–3]

Diagnosis of PHEO in humans is based mainly on biochemical detection of excessive production of the secretory products of the tumor. Widely used tests include measurement of the concentration of catecholamines and their metabolites metanephrine and normetanephrine in 24-hour urine samples, or metanephrines in plasma.[4] Although these tests are regarded as being reasonably sensitive and specific, the diagnosis of PHEO in human medicine remains challenging. Dogs with PHEO might have only mild or inconsistent increases in plasma or urine catecholamines/metanephrines and various diseases and medications can increase catecholamine synthesis and lead to false-positive results.[5–7]

From the Clinic for Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland. Presented in part as oral abstract at the 18th Annual European College of Veterinary Internal Medicine Forum, Ghent, Belgium 2008.

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Submitted February 22, 2010; Revised May 3, 2010; Accepted June 18, 2010.

10.1111/j.1939-1676.2010.0578.x

Evaluation of diagnostic tests for PHEO in veterinary medicine has only recently been carried out.[8] Dogs with PHEO had significantly higher urinary epinephrine, norepinephrine, and normetanephrine to creatinine ratios compared with healthy dogs.[9] Whether other diseases cause an increase in the concentration of urine catecholamines and metanephrines has not been investigated in dogs.

Hyperadrenocorticism (HAC) is 1 of the most important differential diagnoses for PHEO. Both diseases might have similar clinical signs such as weakness, tachypnea, panting, polydipsia/polyuria, hypertension-associated bleeding as well as similar abnormalities of the adrenal glands detected via ultrasonography. Additionally, it is known from humans that glucocorticoids might increase catecholamine production and release.[10] The objective of the study was to determine and compare the concentrations of urine catecholamines and metanephrines in healthy dogs, dogs with HAC and dogs with PHEO.

Materials and Methods

Animals and Criteria for Selection of Cases

Dogs with HAC. Dogs suspected of having HAC underwent a thorough clinical examination, and blood and urine samples were collected for a CBC, biochemical profile, urinalysis, and urine culture. Work-up included a low-dose dexamethasone suppression (LDDS) test and measurement of the urine cortisol to creatinine ratio (UCC) in urine samples collected at home. In addition, the adrenal glands were examined ultrasonographically. All 14 dogs had a positive LDDS test (cortisol 8 hours after 0.01 mg/kg dexamethasone $>1.0\,\mu g/dL$). In 12 dogs UCC was measured in 2 urine samples collected on consecutive days at home and was positive ($>10\times10^{-6}$) in 10 dogs, in 2 dogs UCC was within the reference range. In 1 dog UCC was measured once and was found to be elevated. The following criteria were used for inclusion of dogs in the study: the presence of clinical signs consistent with HAC (polyuria, polydipsia [pu/pd],

polyphagia, dermatological problems, decreased activity, panting, pendulous abdomen); a minimum of 1 positive screening test; agreement of the owner to regular re-evaluations over a 6-month period; and other treatments for HAC (eg mitotane, radiation) had not been instituted.

Pituitary-dependent HAC (PDH) was diagnosed by means of a bilateral symmetrical appearance or bilateral enlargement of the adrenal glands on ultrasonograms. All dogs with PDH underwent therapy with trilostane starting with dosages of 2–5 mg/kg, re-evaluations were performed as reported previously.[11] To reduce the risk that concurrent PHEO was present, only those dogs with well-controlled HAC and no other clinical signs during a minimum of 6 months after starting therapy were used in the study. Ten dogs with PDH, ranging in age from 5 to 13 years (median 9) and weighing 8.2–58.0 kg (median 18.7), were used. Six dogs were male (4 castrated) and 4 were female (3 spayed). Breeds included Hovaward (1), Dachshund (1), Petit Griffon Bleu (1), Tibetan Terrier (1), Giant Schnauzer (1), and five mixed-breed dogs.

HAC caused by adrenocortical tumor (ATH) was diagnosed when a mass was seen via ultrasonography in 1 or both adrenal glands. ATH was confirmed by histological examination of the adrenal gland(s) after adrenalectomy and resolution of clinical signs in 3 dogs; in another dog, the diagnosis was confirmed by postmortem examination. The 4 dogs with ATH (2 unilateral adenoma, 1 bilateral adenoma, 1 carcinoma) were 9, 9.3, 10, and 12 years old and weighed 5, 10, and 32 (2) kg, respectively. Two of them were male, 2 were female (1 spayed), and breeds included Miniature Poodle, West Highland White Terrier, Italian Cattle Dog, and Golden Retriever.

Dogs with PHEO. Seven dogs with PHEO were included; the results of 6 were part of a previous study.[9] Dogs had various clinical signs including weakness, polyuria/polydipsia, tachypnea, tachyarrhythmia, or collapse. Ultrasonography identified unilateral adrenal masses in 6 dogs (left adrenal gland in 4 dogs, right adrenal gland in 2 dogs) and bilateral masses in 1 dog. Diagnosis of PHEO was confirmed by histological examination of the adrenal gland(s) after adrenalectomy or postmortem examination. The dogs were 11–16 years old (median 12) and weighed 2.8–41.8 kg (median 20). Four of them were male (2 castrated) and 3 were spayed females, and breeds included Yorkshire Terrier (1), Cairn Terrier (1), Bearded Collie (1), Boxer (1), Bernese Mountain Dog (1), and 2 mixed-breed dogs.

Healthy Dogs. Ten healthy client-owned dogs were included as controls.[9] The dogs were considered healthy based on information provided by their owners, and the results of physical examination, CBC, biochemical profile, urinalysis, and ultrasonography of the adrenal glands. The dogs were 5–12 years old (median 8.4) and weighed 13.7–41.6 kg (median 26.2). Four were male (1 castrated) and 6 were female (4 spayed), and breeds included Gordon Setter (2), Tervueren (1), Golden Retriever (1), Standard Poodle (1), Australian Shepherd (1), Berger Blanc Suisse (1), and 3 mixed-breed dogs. The use of the dogs had been approved by the ethical committee of the Kanton Zurich (no. 199/2004).

Urine Collection for Measurement of Catecholamines and Metanephrines

In all dogs, voided urine was collected in the hospital after physical examination, blood sampling, and abdominal ultrasonography. In 13 dogs with HAC an additional urine sample was collected by the owner 1 week after discharge to investigate whether the stress of veterinary manipulations affected the results. This second urine sample was collected before initiating treatment with trilostane or before adrenalectomy.

Urine Processing for Measurement of Catecholamines and Metanephrines

Ten milliliters of urine was placed in a plain silicone-coated tube containing 120 μL of 20% hydrochloric acid (HCl). Urinary pH was measured with pH indicator strips (range of pH scale 1–6) and 20% HCl was added to ensure that urine pH was ≤2, as reported previously.[8] Urine collected by owners was protected from light and stored at 4°C for a maximum of 48 hours according to written instructions. The urine container was placed on ice for transportation to the laboratory where it was stored at −20°C until analysis.

Urinary free catecholamines, comprising epinephrine and norepinephrine, and fractionated metanephrines, comprising free and conjugated metanephrine and normetanephrine, were separated and concentrations were determined quantitatively by high pressure liquid chromatography with electrochemical detection with commercial reagents from Bio-Rad[a] as reported previously.[8,9]

The concentrations of urine catecholamine and metanephrine were correlated to the concentration of urine creatinine as reported previously.[8,9]

Statistical Analysis

Data were analyzed by means of nonparametric methods by Statistical Package for the Social Science (SPSS), Software Packets for Windows, Version 11, and GraphPad PRISM for Windows, Version 3.0. Ranges and medians are given. The Wilcoxon signed ranks test and the Mann-Whitney *U*-test were used for comparisons within groups and between groups, respectively. Dogs with PDH and dogs with AT were classified into 1 group, called HAC because of the low number of dogs. Bonferoni's correction was applied and values of $P < .05$ were considered significant.

Results

Comparison of Results in Dogs with HAC, Dogs with PHEO, and Healthy Dogs

Dogs with HAC had significantly higher urinary epinephrine, norepinephrine, and normetanephrine to creatinine ratios than healthy dogs ($P = .001$, .024, .002), whereas metanephrine to creatinine ratios did not differ ($P = .101$). Similar results were obtained when measurements of dogs with PHEO were compared with those of healthy dogs ($P = .008$; .001, .001, .922).

Comparison of results of dogs with HAC and dogs with PHEO revealed that urinary epinephrine, norepinephrine, and metanephrine to creatinine ratios did not differ ($P = .601$, .201, .370). However, the urinary normetanephrine to creatinine ratio was significantly higher in dogs with PHEO ($P = .011$). In 4 of the 7 dogs with PHEO urinary normetanephrine to creatinine ratios were higher than those of dogs with HAC. Using a cut-off ratio of 4 times the highest normetanephrine to creatinine ratio measured in controls, no overlap between dogs with HAC and dogs with PHEO was seen (Table 1, Fig 1a–d).

Comparison of Results of Urine Collected in the Hospital and at Home in Dogs with HAC

Norepinephrine, epinephrine, normetanephrine, and metanephrine to creatinine ratios of urine samples collected in the hospital and those collected at home 1 week after discharge did not differ ($P = .124$, .135, .463, .650) (Fig 2a–d).

Table 1. Ranges and medians of urinary epinephrine, norepinephrine, metanephrine, and normetanephrine to creatinine ratios in healthy dogs, dogs with hyperadrenocorticism (HAC), and dogs with pheochromocytoma (PHEO).

	Epinephrine : Creatinine	Norepinephrine : Creatinine	Metanephrine : Creatinine	Normetanephrine : Creatinine
Healthy dogs	1.0–8.0 (3.5)	4.0–19.0 (8.0)	46.0–255.0 (105.5)	14.0–91.0 (59.5)
Dogs with HAC	2.0–73.0 (11.5)	6.0–95.0 (15.0)	26.0–227.0 (73.0)	53.0–323.0 (117.5)
Dogs with PHEO	4.0–31.0 (8.0)	11.0–40.0 (28.0)	21.0–375.0 (102.0)	157.0–925.0 (414.0)

Discussion

The results of this study indicate that HAC might be associated with increased concentrations of urine catecholamines and metanephrines. Approximately, 50% of dogs with HAC had urinary epinephrine, norepinephrine, and normetanephrine : creatinine ratios above those of control dogs and the difference was statistically significant. Only the urinary metanephrine to creatinine ratio did not differ between the 2 groups of dogs. HAC is 1 of the most common endocrine diseases of dogs and is often associated with characteristic clinical signs, including symmetrical alopecia, thinning of the skin, and polyphagia. However, in some cases, less specific signs such as polyuria/polydipsia, weakness, panting, muscle tremors, or hypertension-associated bleeding dominate,

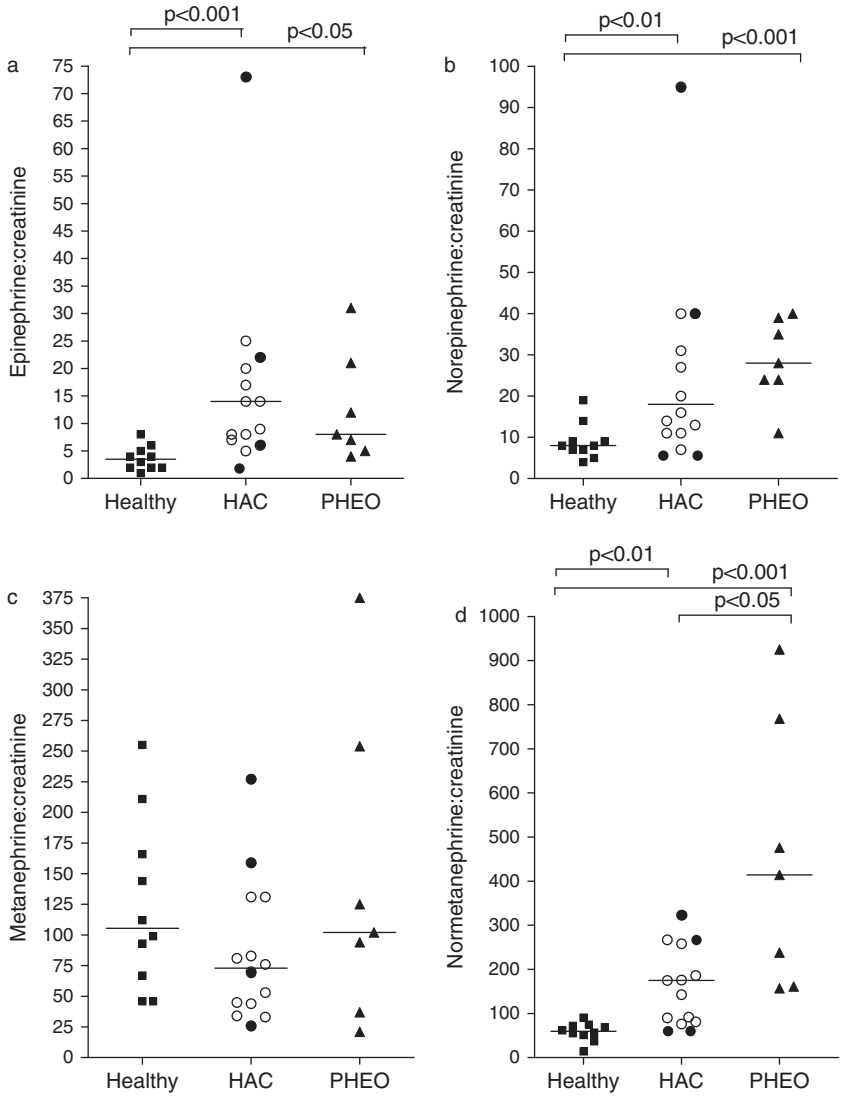

Fig 1. Urinary epinephrine (a), norepinephrine (b), metanephrine (c), and normetanephrine (d) to creatinine ratios in healthy dogs (n = 10, squares), dogs with hyperadrenocorticism (HAC, n = 14) and dogs with pheochromocytoma (PHEO, n = 7, triangles). In dogs with HAC, open circles represent dogs with pituitary-dependent HAC, and closed circles represent dogs with adrenocortical tumor. All urine samples were collected in the hospital. The most important finding was that dogs with PHEO have significantly higher urinary normetanephrine to creatinine ratios than dogs with HAC; see text for further details.

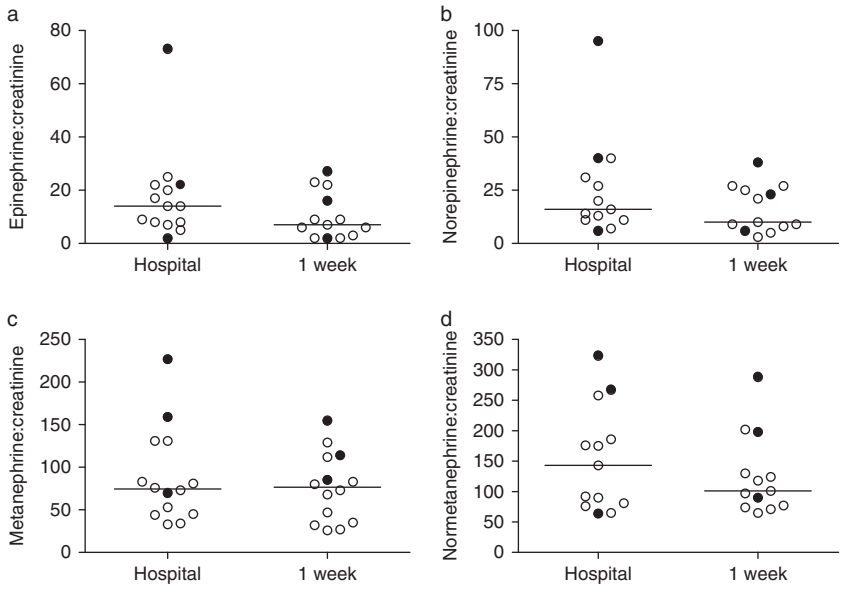

Fig 2. Comparison of urinary epinephrine (a), norepinephrine (b), metanephrine (c), and normetanephrine (d) to creatinine ratios in samples collected from dogs with hyperadrenocorticism (HAC) in the hospital and 1 week later at home by the owner. There was no difference between the 2 types of urine samples with respect to any of the variables. Open circles represent dogs with PDH, and closed circles represent dogs with adrenocortical tumor.

and therefore various other diseases including PHEO must be considered as differential diagnosis. Both HAC and PHEO might be associated with an abnormal ultrasonographic appearance of the adrenal glands. Unfortunately, there is no pattern of echogenicity or architecture which would help to discriminate PHEO from other adrenal masses such as cortisol-producing tumors.[12] PHEO might also be difficult to differentiate from PDH ultrasonographically, especially in cases in which the latter is associated with macronodular hyperplasia.[2] For this reason, our study was not limited to dogs with ATH, but instead included dogs with PDH.

PHEO and HAC (either ATH or PDH) can occur in the same dog.[13,14] We tried to eliminate the risk that our dogs with HAC had concurrent PHEO by documentation of clinical signs after adrenalectomy (combined with diagnostic histological findings) or a conclusive postmortem examination. In dogs with PDH in which histological examination of the adrenal glands was not possible, it was necessary for dogs to be re-evaluated in the hospital at regular intervals and to be clinically well controlled for 6 months after starting trilostane therapy.

Dogs with PHEO have significantly increased urinary catecholamines and normetanephrine to creatinine ratios compared with healthy dogs.[9] While urinary epinephrine and norepinephrine to creatinine ratios overlapped to some degree in healthy dogs and dogs with PHEO, the urinary normetanephrine to creatinine ratio was consistently higher in the latter.[9] The results are in agreement with those in humans with PHEO in which the concentration of urine normetanephrine has the highest sensitivity among the urinary variables.[5,15]

This finding is probably because of the fact that the vast majority of PHEO predominantly produce norepinephrine, which is metabolized into normetanephrine. The latter is continuously released into the circulation, whereas norepinephrine is secreted only intermittently or in negligible amounts.[5,16] According to our preliminary results the situation might be similar in dogs. Only the measurement of the concentration of plasma metanephrines is currently thought to have a higher sensitivity than urine normetanephrine.[17]

Interestingly, the urinary normetanephrine to creatinine ratio was also the only variable that was significantly higher in dogs with PHEO than in dogs with HAC. In human medicine the interactions between glucocorticoids and catecholamines are well known.[10] Glucocorticoids can induce catecholamine synthesis in PC12 pheochromocytoma cell cultures. They can also induce biosynthetic enzymes, such as phenylethanolamine *N*-methyltransferase, which converts norepinephrine to epinephrine, tyrosine hydroxylase, which is the rate-limiting enzyme in catecholamine biosynthesis, and dopamine β-hydroxylase, which converts dopamine to norepinephrine.[10,18–20] Glucocorticoids also increase the release of catecholamines from perfused canine adrenal glands.[21] Humans with cortisol-secreting adrenal tumors have higher concentrations of urine catecholamines and metanephrines than healthy controls, and levels overlap to some extent with those of dogs with PHEO.[5] To distinguish true-positive from false-positive results, the use of cut-off values, usually 2–3 times the upper limit of normal, is recommended.[7,17] A higher cut-off value, for example 4 times normal, is associated with a nearly 100% probability of PHEO.[22] The major remaining diagnostic dilemma occurs in humans with smaller increases (less than 2–3 or 4 times normal)[17] because there is no reliable way to diagnose which of these have PHEO.[6]

The results of the present study indicate that the same problem exists in dogs. By using a cut-off value of 4 times the highest normetanephrine to creatinine ratio measured in healthy controls, no overlap between HAC and PHEO was seen; all dogs with a ratio >364 had PHEO. However, the diagnosis in 3 of the 7 dogs with PHEO would have been missed. Using a cut-off value of 3 times normal, 1 dog with HAC would have been misdiagnosed, and with a cut-off value of twice normal, 4 of 13 dogs with HAC would have been misdiagnosed as having PHEO. In the PHEO group, the diagnosis would have been missed in 3 resp. 2 dogs by using a cut-off value of 3 resp. 2 times normal. We currently suggest to use a cut-off of 4 times normal, however verification in a larger number of cases is needed.

Veterinary care affects the concentrations of urinary catecholamines and metanephrines.[8] Thus, it would appear prudent to collect urine samples at home. This approach has some limitations in dogs with PHEO. In our hospital, immediate therapy with phenoxybenzamine is usually started in dogs suspected of having PHEO. However, phenoxybenzamine increases concentrations of norepinephrine and normetanephrine and is a major cause of false-positive test results in humans.[17] To ensure identical conditions in all 3 groups of dogs, urine was sampled in the hospital after physical examination, blood sampling, and abdominal ultrasonography and before the administration of phenoxybenzamine. This approach also seemed reasonable based on the finding that in dogs with HAC the concentration of urine catecholamines and metanephrines did not differ in urine samples collected in the hospital and at home 1 week after discharge.

In summary, dogs with HAC had increased concentrations of urine catecholamines and normetanephrine. The latter variable, however, was significantly higher in dogs with PHEO than in dogs with HAC. According to our preliminary results a cut-off value of 4 times the upper limit of normal is associated with PHEO. However, with this approach the diagnosis will be missed in some dogs with PHEO. Repetitive testing and/or extensive work-up to exclude other diseases with similar clinical appearance might therefore be required to confirm a diagnosis of PHEO in certain cases.

Footnote

[a] Bio-Rad, Munich, Germany

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