

The Effects of Illness on Urinary Catecholamines and their Metabolites in Dogs

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Background: Urinary catecholamines and metanephrines have been proposed as a diagnostic tool for identifying canine pheochromocytomas, but the effects of critical illness on urine concentrations of catecholamines and metanephrines currently are unknown.

Objectives: To examine the effects of illness on urine concentrations of catecholamines and metanephrines in dogs.

Animals: Twenty-five critically ill dogs and 25 healthy age- and sex-matched control dogs.

Methods: Prospective observational study. Urine was collected from healthy and critically ill dogs, and urine concentrations of epinephrine, norepinephrine, metanephrine, and normetanephrine were measured by high-performance liquid chromatography with electrochemical detection. Urinary catecholamine and metanephrine:creatinine ratios were calculated and compared between groups.

Results: Urinary epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios were higher in critically ill dogs when compared with a healthy control population ($P = .0009$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively).

Conclusions and Clinical Importance: Illness has a significant impact on urinary catecholamines and their metabolites in dogs. Further investigation of catecholamine and metanephrine concentrations in dogs with pheochromocytomas is warranted to fully evaluate this test as a diagnostic tool; however, the findings of this study suggest that the results may be difficult to interpret in dogs with concurrent illness.

Key words: Epinephrine; Metanephrine; Norepinephrine; Normetanephrine.

Pheochromocytomas are uncommon tumors of dogs that pose a diagnostic challenge. These tumors of the chromaffin cells develop within the adrenal medulla or sympathetic ganglia.^{1–4} When functional, pheochromocytomas secrete excessive quantities of catecholamines, leading to a variety of nonspecific, and often paroxysmal, clinical findings including hypertension, tachyarrhythmias, weakness, and collapse.^{2,3,5–8} Prognosis for dogs with invasive tumors is guarded, but surgical excision can be curative.^{2,5,9,10} Clinical findings, blood pressure monitoring, and diagnostic imaging can be used to increase suspicion of a pheochromocytoma, but a convenient and reliable method to confirm the diagnosis is lacking.

With the increasing availability of ultrasonography, incidental adrenal masses are frequently identified. In patients with nonspecific or inapparent clinical signs, it is often uncertain as to how testing should proceed when an adrenal mass is discovered. Pheochromocytomas cannot be distinguished ultrasonographically from adrenocortical tumors and many of the clinical signs seen with pheochromocytomas and hyperadrenocorticism overlap.^{2,3,5,7,8,11,12} Testing for both conditions would seem

prudent in any dog with an adrenal mass. However, at this time, biopsy is the only reliable method for pheochromocytoma diagnosis. Because manipulation of these tumors can lead to massive catecholamine release, such procedures are not without risk.^{5,7,10} The use of nuclear scintigraphy and positron emission tomography for pheochromocytoma diagnosis has been described in the veterinary literature, but the availability of these techniques is limited.^{13,14}

In humans, urine and plasma concentrations of catecholamines (epinephrine and norepinephrine) and metanephrines (metanephrine and normetanephrine) have been used for the routine screening of patients for pheochromocytomas for a number of years. Within the chromaffin cells, there is constant intracellular metabolism of catecholamines to metanephrines, creating stable concentrations that are minimally influenced by extracellular metabolism of circulating catecholamines.^{15,16} In contrast, circulating catecholamine concentrations may vary in response to the secretory pattern of the tumor or to any number of stressors in patients without pheochromocytomas.^{3,15–17} Because of this variability in catecholamine concentrations, measurements of metanephrines are preferred because they have been found to be more sensitive and specific tests for the diagnosis of pheochromocytomas.^{18–23} Urinary catecholamines and metanephrines in humans traditionally have been measured in urine collected over a 24-hour period. However, studies evaluating concentrations in a single voided sample have shown a high degree of sensitivity and specificity in the diagnosis of pheochromocytomas.²⁴

The use of catecholamine and metanephrine concentrations as a diagnostic tool in veterinary medicine has been limited by cost, availability, and the lack of reference intervals for dogs.^{5,6,10,25} A recent study by Kook et al²⁶ found that the urinary normetanephrine:creatinine ratios in 7 dogs with pheochromocytomas were

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significantly higher compared with a population of healthy control dogs. However, the effects of nonadrenal illness on catecholamine and metanephrine concentrations in dogs are largely unknown. Critical illness in humans has been shown to increase plasma catecholamine and metanephrine concentrations,^{27–29} and although similar studies have not been performed in dogs, there is evidence that the stress of hospitalization can increase urinary catecholamines in dogs.³⁰ Fifty to 60 percent of dogs diagnosed with pheochromocytomas have concurrent disease.^{2,3,7} Thus, a better understanding of the magnitude of the effect of nonadrenal illness on these compounds is vital for interpretation of catecholamine and metanephrine concentrations in dogs.

The primary objective of this study was to examine the effects of illness on urine concentrations of catecholamines and metanephrines in dogs. Secondary objectives were to evaluate the effects of storage on the measurement of catecholamines and their metabolites and to provide reference values that may aid in the diagnosis of canine pheochromocytomas. We hypothesized that critically ill dogs would have significantly higher urinary catecholamine:creatinine ratios compared with the control population, but that metanephrine and normetanephrine:creatinine ratios would not be significantly different between the groups.

Materials and Methods

Animals

The study population for this prospective study consisted of 25 critically ill dogs (Group 2) from the Virginia-Maryland Regional College of Veterinary Medicine Veterinary Teaching hospital's intensive care unit and 25 healthy age- and sex-matched control dogs (Group 1). The study was approved by the Virginia Tech Animal Care and Use Committee. All owners signed informed consent. All dogs were > 5 years of age and did not have a history or evidence of congestive heart failure, renal disease, or adrenal disease based on physical examination, a CBC, biochemistry profile, urinalysis, indirect blood pressure measurement, and abdominal ultrasonography or pathologic evaluation of the adrenal glands. On ultrasound examination, the adrenal glands were measured perpendicular to the long axis of the gland. They were considered normal if they were < 8 mm thick and had normal shape, contour, and acoustic texture. Dogs were excluded if they had undergone surgery in the past month or had been given medications within the past 2 weeks that were known to affect catecholamine concentrations or to interfere with the biochemical assay (including phenylpropanolamine, prazosin, sympathomimetics, β -blockers, tricyclic antidepressants, and acetaminophen).^{31–34} Dogs in Group 2 were categorized by the primary disease process contributing to their illness. Case outcome was defined as surviving if the dog was discharged from the hospital and nonsurviving if the dog died or was euthanized while in the hospital. Dogs in Group 1 were determined to be healthy based on the lack of clinically important abnormalities identified on the physical examination and tests described previously.

Experimental Protocol

In all dogs, voided urine was collected to measure epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios. In 8 dogs, 4 urine samples were collected over an 8–14-hour period to evaluate the consistency of urinary excretion of catecholamines and metanephrines throughout the day. This group consisted of 2 dogs

from the critically ill population, 4 dogs from the healthy dog population, and 2 apparently healthy dogs that were excluded from the remainder of the study because of mild bilateral adrenomegaly.

Sample Collection and Processing

Ten milliliters of urine were placed into plain glass tubes containing 197 μ L of 6 M hydrochloric acid, gently mixed, divided into aliquots in polypropylene tubes, and frozen at -70°C . Samples were processed and frozen within an hour of collection. Analysis of urinary catecholamines and metanephrines was performed within 5 weeks of sample collection.

Analysis of Urinary Catecholamines and Metanephrines

High-performance liquid chromatography with electrochemical detection was used to measure urinary free epinephrine and norepinephrine concentrations and total (free plus conjugated) metanephrine and normetanephrine concentrations using commercial reagents^{a,b} in accordance with previously reported methods.^{30,35–37} All samples were run in duplicate. For samples in which there was no recovery of an analyte, the measured value was recorded as the assay's lower limit of detection for that analyte.

The intrassay and interassay coefficients of variation were calculated using a pooled urine sample collected from healthy dogs. Aliquots of this sample were run 8 consecutive times on day 1 and duplicate samples were run 9 additional times over a period of 3 months. Stability of the frozen samples over time was assessed by comparing urine concentrations of catecholamines and metanephrines from fresh urine samples collected on day 1 with samples frozen for 3, 5, 8, 11, 12, 15, 25, 50, and 78 days at -70°C .

Urine creatinine concentrations were measured in unacidified urine samples on the day of collection using a modified Jaffe procedure on an automated chemistry analyzer.^c Urine epinephrine, norepinephrine, metanephrine, and normetanephrine:urine creatinine ratios were calculated.

Statistical Analysis

Normal probability plots were generated to determine if continuous data followed a normal distribution. Subsequently, continuous data were summarized as medians if not normally distributed (epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios), or means if normally distributed (age, body weight, average adrenal gland size, and blood pressure). Contingency tables were generated for categorical data (sex).

Body weight and age were compared between the groups using a 2-sample *t*-test whereas the numbers of male and female dogs were compared between the groups with χ^2 test. The stability of the compounds over a 78-day period was assessed using locally weighted robust scatter plot smoothing. Evaluation of the variability of epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios within individual dogs over the course of a day was tested using mixed model analysis of variance with dog as a random effect. Associations between age, body weight, blood pressure, and average adrenal gland size and the ratios (epinephrine, norepinephrine, metanephrine, or normetanephrine:creatinine ratios) were investigated using scatter plots followed by analysis of covariance. The effect of sex on each of the ratios was tested with the Wilcoxon rank sum test. For dogs in Group 2, the effect of case outcome on each of the ratios was tested with the exact Wilcoxon rank sum test.

Epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios were compared between the 2 groups with Wilcoxon's rank sum tests. Correlations between epinephrine and metanephrine:creatinine ratios and between norepinephrine and normetanephrine:creatinine ratios were assessed with

Spearman's rank correlation coefficients. Reference intervals for the ratios in each of the groups were estimated with the nonparametric percentile method. Commercial software was used to perform all statistical analyses^d and to calculate reference intervals.^e Statistical significance was set to $P < .05$.

Results

Study Population

The healthy group (Group 1) included 12 castrated male and 13 spayed female dogs. The median age was 9 years (range, 5–15 years) and the median body weight was 27.7 kg (range, 4–39 kg). Breeds represented included mixed breed ($n = 14$), Labrador Retriever ($n = 4$), Golden Retriever ($n = 2$), Australian Cattle Dog ($n = 1$), Australian Shepherd ($n = 1$), Black and Tan Coonhound ($n = 1$), Border Collie ($n = 1$), and Norwich Terrier ($n = 1$).

The critically ill group (Group 2) included 11 castrated males, 1 intact male, and 13 spayed females. The median age was 9 years (range, 5–13 years). Median body weight was 16.4 kg (range, 5–40.9 kg). Breeds included mixed breed ($n = 5$), Labrador Retriever ($n = 3$), Shih Tzu ($n = 3$), Beagle ($n = 2$), Dachshund ($n = 2$), Shetland Sheepdog ($n = 2$), Bernese Mountain Dog ($n = 1$), Bichon Frise ($n = 1$), Border Collie ($n = 1$), Boxer ($n = 1$), Cocker Spaniel ($n = 1$), Pomeranian ($n = 1$), Scottish Terrier ($n = 1$), and Weimaraner ($n = 1$). Diagnoses for dogs in Group 2 included immune-mediated disease ($n = 7$), hepatobiliary disease ($n = 4$), pancreatitis ($n = 3$), gastrointestinal disease ($n = 3$), neurologic disease ($n = 3$), neoplasia ($n = 3$), or respiratory disease ($n = 2$). Eight of these dogs had multiple disease processes affecting ≥ 2 body systems but were classified according to the pathologic process that was considered to be the most life-threatening at the time of hospitalization. Twenty dogs in Group 2 survived until discharge from the hospital and 5 died or were euthanized. There were no differences between the groups in regard to sex ($P = 1.0$) or age ($P = .82$), although body weight was greater in Group 1 ($P = .0086$).

All dogs had CBC and serum biochemistry profiles performed. Indirect blood pressure measurements were performed in all dogs, with the exception of 1 dog in Group 2 in which the measurement was inadvertently not recorded. This dog was eliminated from analyses evaluating the effects of blood pressure. One dog in Group 2 did not undergo abdominal ultrasound examination but was euthanized and had grossly and histopathologically normal adrenal glands at necropsy. The right adrenal gland was not identified during ultrasound examination in a second dog in Group 2, but both adrenal glands appeared grossly normal during a subsequent exploratory laparotomy. Urine was collected from all dogs during natural voiding, with the exception of 1 dog in Group 1 in which a cystocentesis was performed to obtain an adequate sample.

Intrassay and Interassay Variability

The intrassay coefficients of variation were 9, 4, 7, and 8% for epinephrine, norepinephrine, metanephrine, and

normetanephrine, respectively. The interassay coefficients of variation were 31, 11, 13, and 11%, respectively, for epinephrine, norepinephrine, metanephrine, and normetanephrine. One duplicate set of samples used to calculate the interassay coefficient of variation had epinephrine concentrations that were double the concentrations measured in all other samples. This pair of readings was considered an outlier, and when it was eliminated from analysis, the interassay coefficient of variation for epinephrine was 9.7%.

Stability of Samples over Time and Variability within Individual Dogs

Locally weighted robust scatter plot smoothing for epinephrine, norepinephrine, metanephrine, and normetanephrine concentrations versus day showed that the compounds were stable over time. Urinary epinephrine, norepinephrine, metanephrine, and normetanephrine: creatinine ratios did not vary in individual dogs over an 8- to 14-hour period ($P = .071$, $P = .15$, $P = .77$, and $P = .64$, respectively) (Fig 1).

Urinary Catecholamines and Metanephrines

Urinary epinephrine:creatinine ratios in Group 2 (median, 6.0 nmol: mmol; range, 1.1–33.2) were greater than in Group 1 (median, 2.12 nmol: mmol; range, 0.52–11) ($P = .0009$) (Fig 2A). Norepinephrine:creatinine ratios in Group 2 (median, 16.3 nmol: mmol; range, 5.9–131) also were higher compared with Group 1 (median, 3.46 nmol: mmol; range, 0.6–12) ($P < .0001$) (Fig 2B). Urinary metanephrine:creatinine ratios in Group 2 (median, 56.9 nmol: mmol; range, 20.6–208) were higher than in Group 1 (median, 23.7 nmol: mmol; range, 7.24–78) ($P < .0001$) (Fig 2C) as were urinary normetanephrine:creatinine ratios (median, 145 nmol: mmol; range, 58.1–1,040 in Group 2; median, 51.8 nmol: mmol; range, 22.5–183 in Group 1) ($P < .0001$) (Fig 2D). Calculated reference intervals for catecholamine and metanephrine:creatinine ratios in healthy and critically ill dogs are presented in Table 1. Urinary epinephrine:creatinine ratios in 4/25 and norepinephrine:creatinine ratios in 16/25 critically ill dogs were higher than the reference intervals calculated for healthy dogs. Metanephrine:creatinine ratios in 9/25 and normetanephrine:creatinine ratios in 10/25 critically ill dogs were above the reference intervals calculated for healthy dogs. Among all samples, there was a positive correlation between urinary epinephrine:creatinine and metanephrine:creatinine ratios ($\rho = 0.83$, $P < .0001$) and between norepinephrine:creatinine and normetanephrine:creatinine ratios ($\rho = 0.92$, $P < .0001$) (Fig 3).

No associations were found between age, body weight, blood pressure, or average adrenal gland size, as measured on ultrasound examination, and epinephrine, norepinephrine, metanephrine, or normetanephrine:creatinine ratios. Female dogs in Group 2 had a median metanephrine:creatinine ratio of 68.9 nmol: mmol (range, 32.2–208) which was greater than the metanephrine:creatinine ratios of male dogs in that group (median, 47.2 mol: mmol; range, 20.6–116) ($P = .042$).

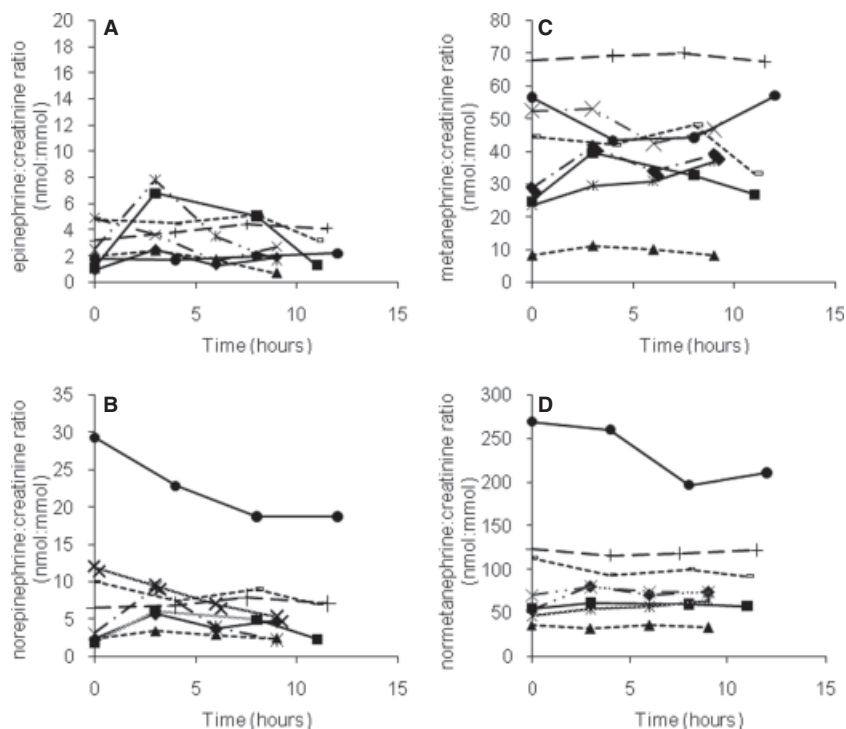


Fig 1. Variability of urinary epinephrine (A), norepinephrine (B), metanephrine (C), and normetanephrine:creatinine (D) ratios in 8 individual dogs measured 4 times over the course of a day. The ratios did not vary significantly in individual dogs over time ($P = .071$, $P = .15$, $P = .77$, $P = .64$ for epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios, respectively).

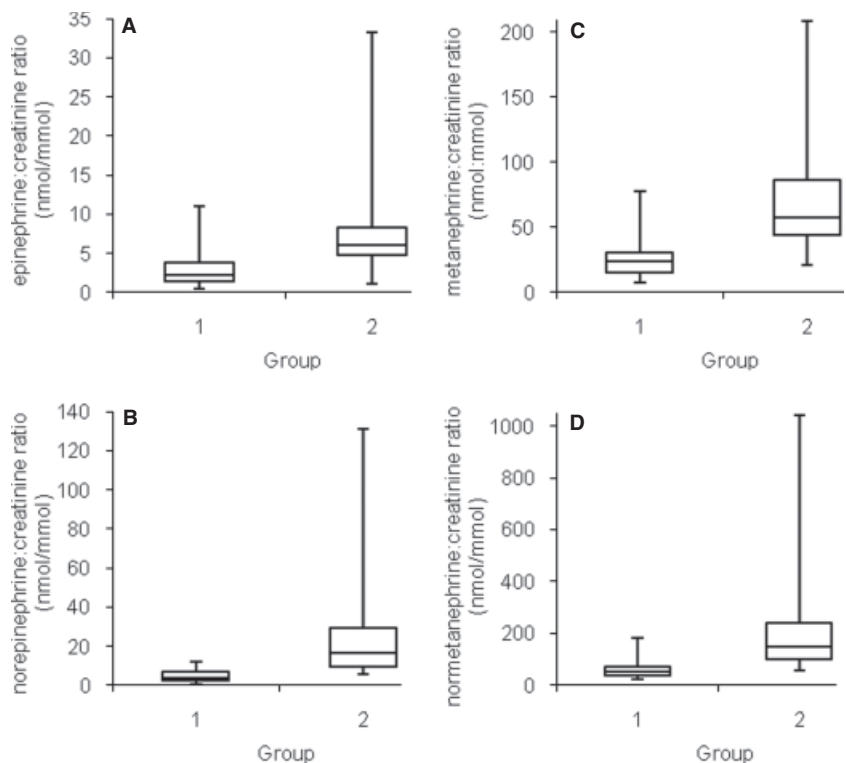


Fig 2. Box and whiskers plots comparing urinary epinephrine (A), norepinephrine (B), metanephrine (C), and normetanephrine:creatinine (D) ratios between healthy dogs (Group 1, $n = 25$) and critically ill dogs (Group 2, $n = 25$). The boxes represent the interquartile ranges, the horizontal bars within the boxes indicate the medians, and the whiskers show the ranges. The ratios differed significantly between groups ($P = .009$ for A, $P < .0001$ for B, C, and D).

Table 1. Calculated reference intervals for urinary catecholamine and metanephrine:creatinine ratios in healthy and critically ill dogs

	Healthy Dogs	Critically Ill Dogs
EPI:creatinine (nmol/mmol)	0.53–10.5	1.1–30.8
NE:creatinine (nmol/mmol)	0.64–11.9	6.0–121
MN:creatinine (nmol/mmol)	7.38–75.9	21.8–204
NMN:creatinine (nmol/mmol)	23.2–178	58.4–941

EPI, epinephrine; NE, norepinephrine; MN, metanephrine; NMN, normetanephrine.

No other significant associations between sex and catecholamine or metanephrine:creatinine ratios were observed. Dogs in Group 2 that died or were euthanized did not have significantly different catecholamine or metanephrine:creatinine ratios than did dogs in Group 2 that survived ($P = .45$, $P = .38$, $P = .89$, and $P = .28$ for epinephrine, norepinephrine, metanephrine, and normetanephrine:creatinine ratios, respectively).

Discussion

The results of this study show that critical illness has a significant impact on urine concentrations of catecholamines and metanephrines in dogs. Activation of the sympathetic nervous system and subsequent release of catecholamines can occur in response to a number of

physiologic and pathologic stimuli. Therefore, the increased urinary catecholamine:creatinine ratios in critically ill dogs were anticipated. The effects of stress on urinary catecholamines have been investigated by Kook and colleagues who found that healthy dogs undergoing routine examinations and blood collection had significantly higher urinary epinephrine and norepinephrine:creatinine ratios on the day of examination compared with urine samples collected 7 days later.³⁰ Similarly, in humans, increased plasma concentrations of catecholamines have been associated with illness in patients suffering from traumatic or vascular brain injury, polysystem trauma, liver failure, hypothyroidism, and myocardial infarction.^{27,28}

Although increased sympatho-adrenal activity is thought to minimally impact metanephrine concentrations,³⁸ this study found that urinary metanephrine and normetanephrine:creatinine ratios were significantly higher in critically ill dogs when compared with a healthy control population. Plasma, and subsequently urine, concentrations of metanephrines are dependent on both intracellular metabolism of catecholamines within the chromaffin cells of the adrenal medulla and catecholamine metabolism that occurs in tissues throughout the body after release of the catecholamines into circulation. The metabolism of circulating catecholamines accounts for only 16–19% of normetanephrine production and 6–10% of metanephrine production, thus increased release of epinephrine and norepinephrine from the adrenal medulla should have a relatively minor impact on total body production of metanephrines.^{38,39} However, these percentages are derived from experimental studies in humans that involved infusions of catecholamines labeled with radiotracers and may not be representative of the physiologic processes that occur during critical illness.

There have been few studies evaluating metanephrine and normetanephrine concentrations in critically ill patients. Chamorro et al²⁹ found humans with acute ischemic stroke and concurrent infection had higher plasma free metanephrine and normetanephrine concentrations than did stroke patients without infection. In the veterinary literature, Kook et al³⁰ observed that even healthy dogs had lower urinary metanephrine and normetanephrine:creatinine ratios at the end of a study in which owners had to collect voided urine samples compared with the start of the study when the dogs were reportedly stressed by the procedure. Most studies that have documented the diagnostic utility of metanephrines are based on study populations of humans suspected of having pheochromocytomas, but this typically does not include patients that are critically ill. The tests largely are performed on an outpatient basis in people with a history of hypertension, suggestive clinical signs, an incidentally discovered adrenal mass, or a genetic predisposition for the neoplasm.^{18,19,21,23,40,41} In a study that included hemodialysis and intensive care unit patients in the control group, 3/45 of the control patients had plasma free metanephrine or normetanephrine concentrations that were >50% above the reference interval.⁴² This observation, as well as the studies by Kook et al³⁰ and

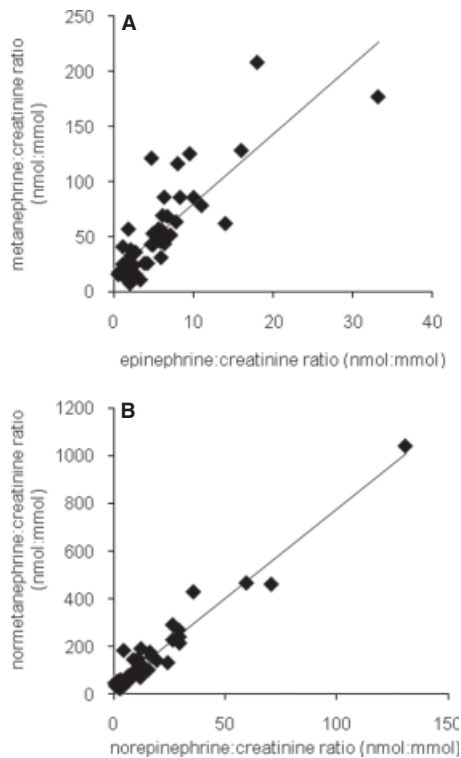


Fig 3. Correlations between urinary epinephrine and metanephrine:creatinine ratios (A) and norepinephrine and normetanephrine:creatinine ratios (B) among all dogs in the study ($n = 50$). Significant positive correlations were observed in both (A) ($\rho = 0.83$, $P < .0001$) and B ($\rho = 0.92$, $P < .0001$).

Chamorro et al,²⁹ are more consistent with the findings of the present study in which critically ill dogs had increased metanephrine and normetanephrine:creatinine ratios. The supposition that the increased metanephrine concentrations were truly because of metabolism of increased quantities of circulating catecholamines is further supported by the very strong positive correlation observed between epinephrine and metanephrine:creatinine ratios as well as between norepinephrine and normetanephrine:creatinine ratios in the dogs in this study.

Measuring concentrations of total metanephrine and normetanephrine rather than free metanephrines also may have contributed to the observed increase in concentrations of metanephrines in critically ill dogs. Total metanephrines include the free and conjugated forms of metanephrine and normetanephrine. Because there is a high concentration of the sulfotransferase isoenzyme, SULT1A3, in the gastrointestinal tract, it is theorized that although the majority of free metanephrine and normetanephrine are formed within the adrenal medulla, the majority of sulfate-conjugated normetanephrine originates from the mesenteric organs through the activity of SULT1A3.⁴³ Because sulfate-conjugated metanephrines typically are 20- to 30-fold higher than free metanephrines,³⁸ the substantial contribution of sulfate-conjugated normetanephrine by the gastrointestinal tract may mask the otherwise stable concentration of free normetanephrine produced by the adrenal glands. This is thought to be 1 explanation for the improved specificity of measuring free plasma metanephrines compared with total urinary metanephrines in the diagnosis of pheochromocytomas in human patients.¹⁸ Critically ill dogs may have increased extra-neuronal production of normetanephrine in their gastrointestinal tract, but because very little epinephrine is produced outside of the adrenal glands, this would not explain the observed increase in metanephrine. Sulfate-conjugated metanephrines are eliminated by the kidneys and are cleared from circulation more slowly than free metanephrines.⁴³ Because patients with renal disease were excluded from this study, decreased renal function in Group 2 would not account for the differences seen between groups.

Until catecholamine and metanephrine concentrations in dogs with pheochromocytomas are fully evaluated, the clinical utility of our calculated reference intervals is limited. Kook et al²⁶ reported 7 dogs with pheochromocytomas and found normetanephrine:creatinine ratios to be the most reliable of the catecholamine and metanephrine:creatinine ratios for distinguishing between healthy dogs and dogs with pheochromocytomas. The range of normetanephrine:creatinine ratios in those dogs was 157–6430 nmol:mmol.²⁶ Although the same assay was used in Kook's study as the study reported here, variation that may exist between laboratories prevents us from making accurate comparisons. However, many of our critically ill dogs had normetanephrine:creatinine ratios within the range reported in dogs with pheochromocytomas. Similarly, in an abstract by Quante et al⁴⁴ there was overlap in the range of normetanephrine:creatinine ratios between

dogs with pheochromocytomas and dogs with pituitary-dependent hyperadrenocorticism. Epinephrine, norepinephrine, and metanephrine:creatinine ratios were not significantly different between the 2 groups. This suggests that urinary metanephrines may not be adequately specific to diagnose pheochromocytomas in dogs with concurrent illness. However, further evaluation of these test results in dogs with confirmed pheochromocytomas may allow identification of a cut-off point above which a pheochromocytoma is likely even in the face of other underlying disease.

One weakness of this study was the use of only abdominal ultrasound examination and historical information to exclude the possibility of adrenal disease in the study population. Retrospective studies of canine pheochromocytomas indicate that only 50–85% of dogs with pheochromocytomas had adrenal masses identified on abdominal ultrasound examination,^{2,7} and it is possible that a small tumor may have gone unnoticed. However, using the gold standard of histopathology to eliminate the possibility of adrenal disease was clearly impractical. Given the rarity of pheochromocytomas, it seems unlikely that occult adrenal neoplasms in the critically ill group would have contributed substantially to the observed outcome.

Groups 1 and 2 were significantly different with regard to body weight. Although age and sex have been shown to affect urinary catecholamine concentrations in humans,⁴⁵ there is no reported effect of body weight. In this study, no correlation was found between body weight and epinephrine, norepinephrine, metanephrine, or normetanephrine:creatinine ratios. Thus, it is unlikely that this difference had an impact on the results.

Intrassay and interassay coefficients of variation were calculated only using pooled urine samples from healthy dogs. Ideally, intrassay and interassay variability would have been evaluated in urine samples with low, normal, and high concentrations to better assess the reliability of the assay over a wide range of values. Unfortunately, because of the large volume of urine required to run repeated samples over time and because we could not predict what the catecholamine and metanephrine concentrations would be before collection, it was not possible to simultaneously collect enough urine from dogs with low, normal, and high concentrations in order to perform this analysis.

In humans, catecholamine and metanephrine concentrations traditionally are measured in urine collected over a 24-hour period. However, studies have shown a high degree of correlation between catecholamine and metanephrine concentrations in a single voided and 24-hour samples, as well as a high degree of sensitivity and specificity of metanephrines in single voided urine samples for the detection of pheochromocytomas.^{24,46,47} The use of catecholamine:creatinine ratios in single voided urine samples is a far more practical technique in veterinary medicine and has been used in previous studies of dogs evaluating urinary catecholamines and metanephrines.³⁰ In the present study, multiple urine samples collected from 8 dogs over the course of the day showed little variation in urine concentrations of catecholamines

and metanephrines. Although only a small group of dogs was evaluated, this further validates the use of single voided samples for the evaluation of urinary catecholamines and metanephrines.

This study shows that illness has a significant impact on urinary catecholamines and their metabolites in dogs. Further investigation of catecholamine and metanephrine concentrations in dogs with pheochromocytomas will be needed to fully evaluate their utility as a diagnostic test for this neoplasm. However, our findings suggest that the results may be difficult to interpret in dogs with concurrent illness. Because many dogs with pheochromocytomas present during a crisis or have an adrenal mass that is incidentally discovered during an imaging procedure to investigate another illness, urinary metanephrines may not be adequately specific to provide an immediate diagnosis. However, testing during a period of disease quiescence or after recovery from an unrelated illness could improve specificity. Furthermore, evaluation of plasma or urinary free metanephrines is warranted to determine if eliminating the effect of sulfate-conjugation might also improve the sensitivity and specificity of metanephrines for pheochromocytoma diagnosis in dogs.

Footnotes

^a Urinary Metanephrines by HPLC, BIO-RAD Laboratories, Hercules, CA

^b Urinary Catecholamines by HPLC, BIO-RAD Laboratories

^c Olympus AU400 Chemistry Analyzer, Beckman Coulter Inc, Brea, CA

^d SAS Version 9.2, SAS Institute Inc, Cary, NC

^e Medcalc, Medcalc Software, Mariakerke, Belgium

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