Influence of Veterinary Care on the Urinary Corticoid: Creatinine Ratio in Dogs

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Physical and emotional stresses are known to increase the production and secretion of glucocorticoids by the adrenal cortex in both humans and experimental animals. The urinary corticoid:creatinine (C:C) ratio is increasingly used as a measure of adrenocortical function. In this study we investigated whether a visit to a veterinary practice for vaccination, a visit to a referral clinic for orthopedic examination, or hospitalization in a referral clinic for 1.5 days resulted in increases of the urinary C:C ratio in pet dogs. In experiment 1, owners collected voided urine samples from 19 healthy pet dogs at specified times before and after taking the dogs to a veterinary practice for yearly vaccination. In experiment 2, 12 pet dogs were evaluated in a similar way before and after an orthopedic examination at a referral clinic. In experiment 3, 9 healthy pet dogs were hospitalized for 1.5 days and urine samples were collected before, during, and after this stay. Basal urinary C:C ratios in all experiments ranged from 0.8 to 8.3 \times 10^{-4}. In experiment 1, the urinary C:C ratio after the visit to the veterinary practice ranged from 0.9 to 22.0 \times 10^{-4}. Six dogs had a significantly increased urinary C:C ratio (responders), but in 5 of these dogs the ratio was \leq 10 \times 10^{-4}. In experiment 2, 8 of 12 dogs responded significantly with urinary C:C ratios ranging from 3.1 to 27.0 \times 10^{-4}. In experiment 3, 8 of 9 dogs had significantly increased urinary C:C ratios, ranging from 2.4 to 24.0 \times 10^{-4}, in some or all urine samples collected during hospitalization. In 4 dogs urinary C:C ratios 12 hours after hospitalization were still significantly higher than the initial values. Thus, a visit to a veterinary practice, an orthopedic examination in a referral clinic, and hospitalization can be considered stressful conditions for dogs. A large variation occurs in response, and in individual dogs the increases in urinary C:C ratios can exceed the cutoff level for the diagnosis of hyperadrenocorticism. Therefore, urine samples for measurement of the C:C ratio in the diagnosis of hyperadrenocorticism should be collected in the dog's home environment, to avoid the influence of stress on glucocorticoid secretion.

Key Words: Clinical examination; Hospitalization; Hyperadrenocorticism; Stress; Urine; Veterinary practice.

Various stresses produce a number of somatic changes, including involution of the thymic lymphatic apparatus, appearance of gastrointestinal ulcers, and enlargement of the adrenal cortex with increased production and secretion of glucocorticoids. Both in humans and in experimental animals, exposure to a variety of alarming stimuli such as surgery, acute infections, burns, agents causing emotional stress, and intense muscular exercise leads to great increases in plasma glucocorticoid concentration and urinary glucocorticoid excretion. The physiologic function of stress-induced increases in plasma glucocorticoid concentrations is to protect not against the source of stress itself, but against the normal defense reactions that are activated by stress, in order to prevent them from overshooting and threatening homeostasis.

Stress-induced changes in plasma glucocorticoid concentrations have also received some attention in companion animal medicine. Willens et al. demonstrated that in cats minor stressors such as handling and skin testing led to a pronounced response by the pituitary–adrenocortical axis. Dogs seem to be less prone to respond to minor stressors such as venepuncture and a novel environment, although immobilization and mild electric foot shocks cause a clear-cut increase in plasma glucocorticoid concentrations. The influence of stress on plasma glucocorticoid concentrations becomes especially important when an abnormality of the pituitary–adrenocortical axis is suspected and the integrity of the system must be tested. Indeed, stress resulting from chronic illness and hospitalization has been reported to possibly influence the results of adrenocortical function tests in humans, cats, and dogs. In recent years measurement of urinary corticoid:creatinine (C:C) ratios has been recognized as an easily performed test of adrenocortical function in dogs. The measurement of urinary C:C ratios is most commonly used as a screening test for hyperadrenocorticism in dogs. Urinary C:C ratios may also be valuable in monitoring the effect of treatment of hyperadrenocorticism, and low urinary C:C ratios may even point to glucocorticoid deficiency. Also, in humans and cats and in cats the C:C ratio of morning or evening urine samples accurately assesses adrenocortical status in patients with hyperadrenocorticism. However, several reports indicate that false-positive results can be found when the urinary C:C ratio is used as a screening test for canine hyperadrenocorticism. In these studies hospitalized dogs were examined or conditions during urine collection were not mentioned. Stress resulting from hospitalization may have resulted in increases in the urinary C:C ratios, which may explain some of the false-positive results in the earlier reports. Because some doubt exists as to whether veterinary care results in increases in urinary C:C ratios, further study of the influence of this type of stress on urinary corticoid excretion is warranted.

To assess the influence of a visit to a veterinary practice for vaccination, a visit to a referral clinic for orthopedic examination, and hospitalization in a referral clinic for 1.5 days, urinary C:C ratios were measured before, during (for hospitalization), and after these interventions in pet dogs.

Materials and Methods

Experiment I

Nine male (4 castrated) and 10 female (6 spayed, 4 anestrous) pet dogs, whose ages ranged from 5 months to 12.3 years (median, 5
years), were evaluated. The sample population consisted of 6 mongrel dogs and 13 purebred dogs of 10 different breeds. The dogs were judged to be healthy according to the information provided by their owners in a detailed questionnaire. Dogs were included when no signs of illness and no treatment during the preceding 3 months were present that could influence urinary corticoid excretion. The owners were requested to evaluate their dogs during the visit to the veterinarian in terms of positive (enthusiastic), negative (fear and flight reactions), or unchanged behavior.

Owners were asked to collect voided urine samples at 36, 24, and 12 hours, and immediately before taking the dogs to a veterinary practice for their yearly vaccination. Subsequently urine samples were collected at 2, 12, 24, and 36 hours after the visit to the veterinarian. The sample collections were completed according to this schedule, except that the 36-hour previst sample was collected in 12 dogs and the 36 hour postvisit sample was collected in the other 7 dogs.

Experiment 2

Five male (2 castrated) and 7 female (2 spayed and 5 between 1 and 3 months after estrus) pet dogs, whose ages ranged from 6 months to 15.3 years (median, 2.7 years), were evaluated. Two mongrel dogs and 10 purebred dogs of 9 different breeds were included. All dogs had orthopedic problems for which they were referred to the Utrecht University Clinic for Companion Animals for the first time. None of them had received treatment that could influence urinary corticoid excretion in the preceding 4 weeks. Owners were asked to observe their dogs during the visit to the referral clinic and to evaluate their behavior as positive (enthusiastic), negative (fear and flight reactions), or unchanged.

On 3 consecutive days, owners collected 7 voided urine samples: 2 on the 1st day (8:00 AM and 8:00 PM), 3 on the 2nd day (between 6:00 and 8:00 AM at home, between 11:00 AM and 1:00 PM at the referral clinic after the orthopedic examination, and at 8:00 PM back home), and 2 on the 3rd day (8:00 AM and 8:00 PM).

Experiment 3

One spayed female and 8 male pet dogs, whose ages ranged from 8 months to 8.7 years (median, 4.9 years), were evaluated. The sample population consisted of 1 mongrel dog and 8 purebred dogs of 7 different breeds. Dogs were included when no signs of illness and no treatment had occurred during the preceding 6 weeks that could influence urinary corticoid excretion, and when they had not previously visited the Utrecht University Clinic for Companion Animals.

Voided urine samples were collected at 8:00 AM and 6:00 PM on 4 consecutive days. In 6 dogs 2 additional urine samples were collected at 1:00 PM on the 2nd and 3rd days. After the collection of the urine sample at 8:00 AM on the morning of the 2nd day, the dog was taken to the referral clinic and housed in an individual cage. The dog stayed overnight and was taken home after collection of urine sample at 6:00 PM on the 3rd day. During the hospitalization period each dog was walked and fed at hours similar to its home routine, and received the same food as at home.

Determination of the Urinary C:C Ratio

The urinary corticoid concentration was measured by radioimmunoassay as described previously.17 The interassay coefficient of variation was 10% and the sensitivity was 1 nmol/L. The urinary corticoid concentration was related to the urinary creatinine concentration (Jaffé kinetic method, initial rate reaction) and C:C ratios were calculated.17,23

Calculations and Statistics

For each dog, the 90% confidence interval of the mean, calculated from the C:C ratios in the urine samples collected before the dogs left their home environment (basal urinary C:C ratios), was determined using the formula mean ± 1.645 × SD. When the C:C ratio in the next urine sample (or samples in experiment 3) exceeded the upper limit of the confidence interval the dog was categorized as a responder. Although exceeding the maximum C:C ratio (8.3 × 10⁻⁶) found in a large population of healthy pet dogs (n = 88),16 the originally introduced cutoff value of 10 × 10⁻⁶ was maintained for the judgment of whether elevations had been induced.11,29

Analysis of variance and unpaired Student's t-tests were used to examine the significance of differences in gender, age, and increment in the positive, negative, and unchanged behavior groups in experiments 1 and 2.11,29 The effect of age on the increment was investigated by calculating linear correlation coefficients (r) and their significance levels in all experiments.11,29 The increment was defined as the difference between the C:C ratio in the 1st urine sample collected after the visit to the veterinary practice or the referral clinic and the mean basal urinary C:C ratio (experiments 1 and 2), and as the difference between the mean C:C ratio in all urine samples collected during hospitalization and the mean basal urinary C:C ratio (experiment 3). A P value <.05 was considered significant.

Results

Experiment 1

The basal urinary C:C ratios ranged from 0.8 to 8.3 × 10⁻⁶ and the C:C ratios in urine samples collected 2 hours after the visit to the veterinary practice ranged from 0.9 to 22.0 × 10⁻⁶ (median, 3.6 × 10⁻⁶). In 13 dogs the urinary C:C ratio did not change significantly, whereas 5 of the 6 responders had a mild increase in the urinary C:C ratio to no higher than 10 × 10⁻⁶ (increments ranged from 1.5 to 5.7 × 10⁻⁶) (Fig 1). Only 1 dog had a pronounced response, with a urinary C:C ratio of 22.0 × 10⁻⁶ and an increment of 17.6 × 10⁻⁶.

According to the owners, 12 dogs (including all the responders) had negative behavior during the visit to the veterinary practice, whereas behavior was positive in 3 dogs and was unchanged in 4. No significant differences were detected in age, gender, or increment between the 3 groups. The effect of age on the increment was insignificant (r = .28).

Fig 1. Urinary corticoid:creatinine (C:C) ratio measured in 19 healthy pet dogs before and after a visit to a veterinary practice for yearly vaccination. The arrow indicates time of visit to the veterinary practice.
caused by an additional visit to a veterinary practice. Dogs before and after a visit to a referral clinic for orthopedic examination. The arrow indicates time of visit to the referral clinic. The increase in the urinary C:C ratio on day 3 in 1 dog was most probably caused by an additional visit to a veterinary practice.

Experiment 2

Basal urinary C:C ratios ranged from 1.6 to $6.1 \times 10^{-6}$, whereas the urinary C:C ratios after the orthopedic examination ranged from 3.0 to $27.0 \times 10^{-6}$ (median, $3.9 \times 10^{-6}$). Four nonresponders and 8 responders had responses varying from mild (urinary C:C ratios between 3.1 and $8.5 \times 10^{-6}$, $n = 6$) to pronounced (urinary C:C ratios of 20.0 and $27.0 \times 10^{-6}$, increments of 17.5 and $20.9 \times 10^{-6}$, $n = 2$) (Fig 2). One dog had a significant increase in the urinary C:C ratio on day 3, which was probably caused by an additional visit to a veterinary practice on that day.

Seven dogs had negative behavior (including the 2 dogs with pronounced responses) and 5 had positive behavior (4 of which were responders) according to their owners. Differences in age, gender, and increment between the 2 groups were insignificant. The increment increased significantly with age ($r = .68$), but after excluding 1 very old dog from analysis the age effect on the increment became insignificant ($r = .47$).

Discussion

The basal urinary C:C ratios found in the pet dogs evaluated were in accordance with those in earlier reports. A large variation occurred in response to the stress situations, ranging from no response at all to a pronounced increase in the urinary C:C ratio. In each of the experiments several dogs responded to the potential stressor, which justifies the conclusion that these 3 aspects of veterinary care can be considered stressful conditions for dogs. The number of responders was lowest after visiting the veterinary practice for vaccination, and highest during hospitalization, which could imply that the severity of the stressor increased from experiment 1 to 3, or that the response also depends on the duration of the stressor.

In cats, cows, and lambs, physical and emotional stress have been reported to result in elevated plasma cortisol concentrations and urinary corticoid excretion. In 1 report even simple veterinary procedures, such as rectal palpation and intramuscular injection, were concluded to increase adrenocortical activity in cows, although in this species considerable animal-to-animal variation exists in response. This is in accordance with our results.

Low specificity has repeatedly been reported to be a major limitation of measurements of urinary C:C ratios in the diagnosis of hyperadrenocorticism. Although high urinary C:C ratios are not specific for hyperadrenocorticism, they are a very accurate reflection of adrenocortical function, which may be increased as a result of stress from nonadrenocortical disease and veterinary care. In the present study, in 6 of 22 responders urinary C:C ratios were above the cutoff value for the diagnosis of hyperadrenocorticism. During hospitalization a significant increase occurred in the urinary C:C ratio in 8 of 9 dogs. These findings can explain some of the false-positive results in earlier reports. Thus, in individual dogs misdiagnosis of hyperadrenocorticism may result from stressful sampling conditions when urinary C:C ratios are used in the diagnosis of this disease.

We found no significant differences in age or gender between the positive, negative, or unchanged behavior groups. The negative behavior group did not have a significantly larger increase in urinary C:C ratio as determined by the increment, compared to the other 2 groups. In experiment 1, all responders had negative behavior, whereas in experiment 2, 4 responders had positive behavior, and 4 had negative behavior. Thus, the type of behavior as described by
the owners did not predict the response in urinary C: C ratio to the stress of veterinary care. Some of the dogs with negative behavior may have had a high plasma cortisol peak of such short duration that it was not reflected in the urinary C: C ratios, which are an integration of corticoid production over a longer period of time. However, this is not a very likely supposition because urinary C: C ratios have proven to be sensitive indicators for the consequences of environmental changes in animals.\(^1\)\(^2\) Also, the behavior was judged by owners, and was therefore subjective. Further studies with ethological variables may be needed to conclude whether certain types of behavior may be preferentially associated with a response of the pituitary–adrenocortical axis.

A significant increase in the increments in urinary C: C ratios with age was found only in experiment 2. This was mainly due to the pronounced response in 1 very old dog, which supports earlier findings that senescence is associated with pituitary–adrenocortical hyperresponsiveness.\(^3\)\(^4\)

Based on the results, a visit to a veterinary practice, an orthopedic examination in a referral clinic, and hospitalization can be considered stressful conditions for dogs. A large variation in response occurred, and in individual dogs large increases in urinary C: C ratios that can exceed the cutoff level for the diagnosis of hyperadrenocorticism may occur. Therefore, collection of urine samples for the diagnosis of hyperadrenocorticism should take place in the dog’s home environment to avoid the influence of stress on glucocorticoid secretion.

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