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Intra- and interindividual variation in urine osmolality and urine specific gravity in healthy pet dogs of various ages

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Abstract

Urine specific gravity (Usg) and urine osmolality (Uosm) are used routinely to assess renal concentrating ability, but limited data on these variables are available for healthy dogs. Consequently, we studied the intra- and interindividual variations in Usg and Uosm in healthy dogs as well as the influence of age and gender on these variables.

Dogs were selected for health and anestrus in females through the use of a detailed questionnaire. Eighty-nine owners collected morning and evening urine samples from their dogs on 2 consecutive days. In 8 dogs in which the Uosm of different samples varied more than 50%, owners collected urine for 24 hours at 2-hour intervals during the day and at 4-hour intervals at night. The possible effect of changes in adrenocortical function with age was assessed by measurements of urinary corticoid/creatinine ratios.

Among all samples, Uosm ranged from 161 to 2830 mOsm/kg and Usg from 1.006 to >1.050. In the morning, Uosm (1541 ± 527 mOsm/kg, range 273 – 2620 mOsm/kg) and Usg (1.035 ± 0.010, range 1.009 - >1.050) were higher than in the evening (Uosm: 1400 ± 586 mOsm/kg, range 161 – 2830 mOsm/kg; Usg: 1.031 ± 0.012, range 1.006 - >1.050). The interindividual coefficient of variation in Uosm was 34.2% for morning urine samples and 41.9% for evening samples. In 8 dogs with large differences in urine concentration, there were 2- to 3-fold increases or decreases in Uosm during the day, and the intraindividual coefficient of variation was 33.0%. There was no relation between gender and urine concentration. Urine concentration in both the morning and evening samples decreased with age. Urinary corticoid/creatinine ratios did not change with age.

It can be concluded that Uosm and Usg vary widely among healthy dogs. Urine concentration is generally lower in the evening than in the morning and is not related to gender. Urine concentration decreases with age, and this cannot be ascribed to an associated increase in endogenous corticoids.

In some dogs, Uosm varies widely during the day, with an intraindividual coefficient of variation approaching the interindividual coefficient of variation. This may be regarded as biologic variation but also could represent an early undiagnosed clinical abnormality.
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Introduction

Urinary concentrating capacity depends on the ability of the hypothalamic osmoreceptors to respond to changes in plasma osmolality, the ability of the atrial and carotid bifurcation baroreceptors to respond to changes in blood pressure or blood volume, and the release of the antidiuretic hormone vasopressin (VP) from the neurohypophysis. In addition, medullary hypertonicity must be generated and maintained, and there must be an adequate number of functional nephrons with an appropriate response to VP (Chew and DiBartola 1989, Reeves and Andreoli 1992).

In the healthy dog, urine osmolality (Uosm) values as low as 50 mOsm/kg are reached after infusion of large quantities of water, i.e., in the absence of VP (Schrier and Berl 1972, Schrier et al. 1972, Anderson et al. 1974, Cadnapaphornchai et al. 1974). In states of dehydration, urine specific gravity (Usg) and Uosm are increased to as much as 1.076 and 2738 mOsm/kg, respectively (Hardy and Osborne 1979).

Urine specific gravity and Uosm are used to assess the ability of the renal tubules to concentrate or dilute glomerular filtrate (Stevens and Osborne 1974). Randomly collected urine samples from normally hydrated dogs are reported to have Usg values ranging from 1.015 to 1.045, whereas Uosm values usually vary between 500 and 1200 mOsm/kg (Bloom 1960, Doxey 1971, Osborne et al. 1972, Coles 1974, Stevens and Osborne 1974, Bush 1975, Bovee 1984).

There appears to be a high degree of individual biologic variability in Usg or Uosm values of urine samples obtained at different times of the day from the same dog (Bovee 1969, Chew and Dibartola 1989). Therefore, a single urine sample may be misleading, and a second sample or a series of samples should be obtained to confirm any abnormality (Doxey 1971, Bush 1975, Bovee 1984). A Usg value of 1.025 or more has been considered evidence of adequate renal concentrating ability (Hardy and Osborne 1979, Finco 1989).

Over the years, these statements, which have appeared in textbooks and which are based on clinical experience and not substantiated by investigatory data, have been used as guides for the assessment of urinary concentrating ability. Except for one report of 20 dogs (Hardy and Osborne 1979), there has been no systematic study of Uosm and Usg in healthy pet dogs. Also, the effects of gender and age on Usg and Uosm values have not been investigated in normal pet dogs.

This study was designed to investigate the influence of age and gender on Uosm and Usg and the fluctuation of these variables during the day in healthy pet dogs. Aged dogs have an elevated basal hypothalamic-pituitary-adrenocortical activity characterised by increased concentrations of adrenocorticotropic hormone (ACTH) and cortisol in plasma and increased urinary corticoid excretion
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(Rothuizen et al. 1993). Consequently, we also measured urinary corticoid/creatinine (C/C) ratios in the dogs in this study to evaluate the possible effect of increased corticoid production on renal concentrating ability (Joles et al. 1980, Biewenga et al. 1991).

Materials and Methods

Animals

Thirty-four male (5 castrated) and 55 female (27 spayed) dogs, ranging in age from 6 months to 15.4 years, were investigated. The sample population consisted of 21 mongrel dogs and 68 pure-bred dogs comprising 31 different breeds. Among the breeds represented were 8 Labrador Retrievers, 5 Golden Retrievers, 5 Bouviers des Flandres, and 5 Border Collies. Other breeds were represented by 4 or fewer dogs.

The dogs were judged to be healthy according to the information provided by their owners in a detailed questionnaire. Dogs were included when there had been no signs of polyuria, polydipsia, or urinary incontinence during the past 3 months and when there had been no signs of illness or any treatment that could influence urinary concentrating ability. Furthermore, only anestrous female dogs were included. The questionnaire also provided information on the availability of food during the day and the composition of the food.

Urine collection

On 2 consecutive days, owners collected 4 urine samples by free catch: 2 in the morning during the first walk and 2 in the evening during the last walk, resulting in 4 urine samples per dog. In 8 dogs with variations in Uosm values of >50%, the owners collected urine at 2-hour intervals during the day and at 4-hour intervals at night for a period of 24 hours.

Methods

In all urine samples, Usg was measured in duplicate by refractometry (Clinical table refractometer, Atago, Tokyo, Japan). Urine osmolality was determined in duplicate by freezing point depression (Cryostatic osmometer 030, Gonotec GmbH, Berlin, Germany). One urine sample from each dog was examined for the presence of glucose by a dipstick method (TES-TAPE, Eli Lilly and Co, Indianapolis, USA). In 176 morning urine samples and in the urine samples collected during 24 hours in 4 dogs, C/C ratios were determined as described previously (Stolp et al. 1983, Rijnberk et al. 1988).
Statistical analysis

Values are expressed as mean ± SD. The significance of changes in Uosm, Usg and C/C ratio with age was examined by linear regression analysis. The interindividual variation in Uosm of morning and evening urine samples and the intraindividual variation in Uosm of urine samples collected during 24 hours were expressed by coefficients of variation. Inter- and intraindividual differences were tested with unpaired and paired, two-tailed Student's t-tests, respectively. A P value <0.05 was considered significant. In 27 dogs, Usg of one or more samples exceeded the upper scale limit of the refractometer (1.050), and Usg values of these samples were not used for further analysis.

Figure 1. Relation between age and urine osmolality (Uosm) in 89 healthy pet dogs, in which Uosm was measured on 2 consecutive days in morning samples (left panel) and evening samples (right panel). Regression lines ± 2SD are shown:
Y = 1809 - 50.0X, r=0.36 (P<0.001), n=178 (left panel)
Y = 1764 - 67.8X, r=0.44 (P<0.001), n=178 (right panel)
Results

The Uosm of morning samples ranged from 273 to 2620 mOsm/kg, whereas the Uosm values of evening samples ranged from 161 to 2830 mOsm/kg. The Uosm of morning samples (1541 ± 527 mOsm/kg) was significantly higher than that of evening samples (1400 ± 586 mOsm/kg) (P<0.001). Neither the Uosm of morning samples of 2 consecutive days nor the Uosm of 2 evening samples differed significantly. The interindividual coefficients of variation for Uosm of morning and evening samples were 34.2% and 41.9%, respectively. The Uosm of both morning and evening samples decreased significantly with age (P<0.001, Figure 1).

The morning Uosm of male (1522 ± 427 mOsm/kg) and female (1553 ± 539 mOsm/kg) dogs did not differ significantly. Also, the Uosm values of evening samples of male (1309 ± 531 mOsm/kg) and female (1456 ± 562 mOsm/kg) dogs were not significantly different.

The Usg of morning samples of all dogs ranged between 1.009 and >1.050 and that of the evening samples between 1.006 and >1.050. The 27 dogs in which Usg of one or more samples was >1.050 were significantly younger than the remaining 62 dogs (P<0.001). After excluding the samples of these 27 dogs, analysis of data from the remaining 62 dogs showed a significant decrease in Usg of the evening samples with age (P<0.001, Figure 2), but no relationship was found between age and Usg of morning samples. In these 62 dogs, the Usg of the morning samples (1.035 ± 0.010) was significantly higher than the Usg of the evening samples (1.031 ± 0.012) (P<0.001). Neither the Usg of the 2 morning samples nor the Usg of the 2 evening samples differed significantly.

**Figure 2.** Relation between age and urine specific gravity (Usg) in 62 healthy pet dogs, in which Usg was measured in evening samples on 2 consecutive days. The regression line ± 2SD is shown:

\[ Y = 1.037 - 0.0009X, r=0.31 \]

(P<0.001), n=124
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In the 8 dogs that were sampled frequently because of the large variation in Uosm of different samples, 2- to 3-fold increases or decreases in Uosm often occurred within 2 hours (Figures 3 and 4). After a meal, Uosm decreased as often

Figure 3. Fluctuation of urine osmolality (Uosm, upper panel) and the urinary corticoid/creatinine ratio (C/C ratio, lower panel) during the day in a 9.5-year-old castrated male schnauzer (closed circle, dotted arrow) and a 7.5-year-old female Belgian shepherd (open square, uninterrupted arrow). Arrows indicate feeding times.

Figure 4. Fluctuation of urine osmolality (Uosm, upper panel) and the urinary corticoid/creatinine ratio (C/C ratio, lower panel) during the day in a 2.5-year-old male Border collie (closed circle, dotted arrow) and a 12.5-year-old castrated female longhaired German pointer (open square, uninterrupted arrow). Arrows indicate feeding times.

In the 8 dogs that were sampled frequently because of the large variation in Uosm of different samples, 2- to 3-fold increases or decreases in Uosm often occurred within 2 hours (Figures 3 and 4). After a meal, Uosm decreased as often
as it increased. The mean intraindividual coefficient of variation for Uosm was 33.0%.

The C/C ratios measured in the morning samples of 88 dogs ranged between 0.3 x 10^{-6} and 8.3 x 10^{-6}, with a mean of 2.9 x 10^{-6} (± 1.4 x 10^{-6}). The mean of the C/C ratios measured in the 2 morning samples did not change significantly with age (Figure 5). In the dogs with large intraindividual variations in Uosm in which measurements were made frequently during 24 hours, the C/C ratios varied with Uosm in some, whereas in others the ratios changed very little (Figures 3 and 4). Glucose was not found in any of the urine samples examined.

**Figure 5.** Relation between age and the mean urinary corticoid/creatinine (C/C) ratio in 2 consecutive morning samples from 88 healthy pet dogs.

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**Discussion**

In 1775, De Beaumarchais (French author, 1732-1799) stated, "Boire sans soif ... c'est ce qui distingue l'homme des autres animaux." (Drinking without thirst ... is what distinguishes man from other animals.) Similar statements have been made by Adolph (1939, 1947, 1950) and Guyton (1991). Adolph (1939, 1947, 1950) demonstrated that after water deprivation, dogs do not drink an amount greater than the measured deficit: "In no instance is water ingested voluntarily in an excess sufficient to give rise to water diuresis, and in no instance is water excreted in a diuresis that gives rise to water drinking" (Adolph 1950).

In contrast to these statements and reports, we found large intra- and interindividual variations in Uosm and Usg in healthy pet dogs. The ranges of Usg (1.006 - >1.050) and Uosm (161 - 2830 mOsm/kg) found among the 89 dogs
examined are also considerably larger than those reported in current veterinary textbooks for normally hydrated dogs (Bloom 1960, Doxey 1971, Osborne et al. 1972, Coles 1974, Stevens and Osborne 1974, Bush 1975, Bovee 1984).

There are very few publications addressing Uosm as a measure of renal concentrating ability (Bovee 1969). Hardy and Osborne (1979) found Uosm values ranging from 976 to 2546 mOsm/kg and Usg values ranging from 1.023 to 1.064 in 20 healthy experimental dogs with free access to water. Our results exceed these values at both ends of the range.

The differences in Uosm and Usg between morning and evening samples were significant, whereas those between the morning or evening samples of 2 consecutive days were not. The lower values in the evening urine samples could reflect differences in water intake between day and night.

A significant decrease in Uosm and in evening Usg occurred with aging. It has been reported that Usg and Uosm have excellent correlation (Hendriks et al. 1978, Van Vonderen et al. 1995), so a significant decrease in morning Usg with age also would have been expected. The absence of such a decrease may have resulted from the fact that Usg values in 27 younger dogs could not be used in the analysis because they exceeded the upper scale limit of the refractometer (1.050). Because Usg was higher in the morning than in the evening, omission of these high Usg values presumably had a great effect on the mean morning Usg, causing the decrease of morning Usg with age to be insignificant.

Rothuizen et al. (1993) described an elevated basal hypothalamic-pituitary-adrenocortical activity in aged dogs, characterised by increased concentrations of ACTH and cortisol in plasma and increased urinary corticoid excretion. In the present study, no effect of age on the C/C ratio was observed. A possible explanation for this discrepancy could be the fact that in the study by Rothuizen et al. (1993), samples were not collected at home but in the clinic (i.e., under stressful circumstances). In addition, the dogs used in the study by Rothuizen et al. (1993) were all relatively old (11 - 15 years). Our results agree with the range of C/C ratios in healthy dogs reported by Stolp et al. (1983). Thus, in the age range studied there was no evidence of increased adrenocortical function and, as a consequence, the decrease in urinary concentrating ability cannot be ascribed to increased corticoid production.

Urinary concentrating ability decreases in senescent humans (Lindeman et al. 1966) and rats (Bengele et al. 1981, Beck et al. 1982). Apart from basal renal concentrating ability, maximum Uosm after water deprivation and that obtained after VP administration have been shown to decrease with advancing age in the rat (Miller 1985, Corman and Michel 1987, Geelen and Corman 1992). Furthermore, there is an increase in VP secretion with age in the rat (Frolkis et al. 1982, Fliers and Swaab 1983). These findings provide evidence that impaired renal
concentrating ability during aging in the rat is not a consequence of decreased VP release from the neurohypophysis, but rather a result of an age-associated reduction in renal responsiveness to VP (Bengele et al. 1981, Miller 1985, Geelen and Corman 1992). Increased secretion of VP in the aging rat could also be a consequence of impaired renal concentrating ability, with water loss and resultant hypovolaemia leading to stimulation of VP release (Miller 1985). Conversely, it is possible that increased VP secretion is a primary event, resulting from aging effects on the central nervous system and hypothalamus, leading to downregulation of renal membrane receptors for VP (Miller 1985).

Although VP secretion also increases with aging in humans (Kirkland et al. 1984, Lucassen et al. 1994), impaired renal concentrating ability has not been reported to be caused by a decrease in the renal response to VP, but rather by a decrease in glomerular filtration rate (GFR) and renal blood flow (RBF) during aging (Lewis and Alving 1938, Lindeman et al. 1960). It is indeed well established that GFR and RBF decrease with age in humans (Hollenberg et al. 1974). Furthermore, it has been demonstrated in dogs (Levinsky et al. 1959) as well as in humans (Levitt et al. 1958, Gullick and Raisz 1960) that a decline in GFR leads to a decrease in urine concentration.

In some dogs, a large intraindividual variation in urine concentration was found. Two- to 3-fold increases or decreases in Uosm often occurred within 2 hours. In these dogs, there was no relationship between urinary corticoid excretion and Uosm. A possible explanation for fluctuation in Uosm during the day could be the effect of activity on drinking behaviour. During the day drinking is associated with the activity of dogs, and water intake is larger in active dogs than in quiet dogs (O’Connor 1975, Golob et al. 1977), although drinking stops before the ingestion of enough water to cause a water diuresis (O’Connor 1975).

Another factor responsible for the fluctuation in Uosm during the day could be the effect of feeding on drinking behaviour. Some researchers have found a very close temporal relationship between eating and drinking (Adolph 1939, Ardisson et al. 1975, Fitzsimons 1979). At least 70% of the total intake of water is consumed just before, during, and immediately after meals (Fitzsimons 1979). Golob et al. (1977) reported that the amount of protein and carbohydrate in the meal is the major factor determining the postprandial water intake. Other reports indicate, however, that feeding and drinking evolve according to independent circadian rhythms (Ardisson et al. 1975, Fitzsimons 1979). In our dogs, Uosm decreased as often as it increased after a meal, so that it is unlikely that the fluctuation in Uosm during the day can be explained solely by the effect of feeding on drinking behaviour.

In dogs, an early satiation of thirst occurs during drinking before any changes are detected in plasma osmolality, plasma volume, or blood pressure as a
result of absorption of water (Adolph 1939, 1950, Thrasher et al. 1981). This anticipatory response protects the dog from drinking an amount of water that would exceed its physiological need (Salata et al. 1987). Furthermore, plasma VP concentration decreases significantly in dogs within 6 minutes after drinking, in the absence of changes in plasma composition (Thrasher et al. 1981, 1987). Similar findings have been reported in humans (Salata et al. 1987, Geelen et al. 1984). Stimulation of oropharyngeal receptors by the ingestion of water probably leads both to the initial inhibition of VP secretion and the temporary satiety after drinking (Thrasher et al. 1981, Geelen et al. 1984, Salata et al. 1987, Thrasher et al. 1987). Nevertheless, our observations indicate that these mechanisms do not prevent all dogs from drinking excessive amounts of water. In some dogs, drinking behaviour led to large fluctuations in Uosm, although apparently not to such a degree that the owners perceived the dogs to be polydipsic or polyuric. It may very well be that in more pronounced cases the iatrotropic threshold is surpassed, and the animals are presented to the veterinarian because of polyuria and polydipsia.

It does not seem appropriate to present reference values for Uosm and Usg in dogs, because the range found in healthy dogs is extremely wide. A low Usg in one urine sample does not exclude the possibility of finding a high Usg in another sample. Therefore, a low Usg does not automatically indicate polyuria and polydipsia of pathological origin. Whenever the suspicion of polyuria and polydipsia arises, urine samples should be collected every 2 hours to measure Uosm. Also in humans it is now recognised that Uosm should be established by repeated measurements, and that this approach may prevent further clinical evaluation (Mevorach et al. 1995).

It can be concluded that Uosm and Usg vary widely among dogs. In general, Uosm is lower at night than in the morning. In some dogs, Uosm varies widely during the day, with an intraindividual coefficient of variation approaching the interindividul coefficient of variation. There are no differences in Uosm values related to gender. The decrease in Uosm with age cannot be ascribed to an associated increase in corticoid production.

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