Effect of strenuous exercise on urine concentrations of homovanillic acid, cortisol, and vanillylmandelic acid in sled dogs

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Objective—To determine whether prolonged exercise by conditioned sled dogs affects urine concentrations of homovanillic acid (a metabolite of dopamine), vanillylmandelic acid (a metabolite of norepinephrine and epinephrine), and cortisol.

Animals—24 conditioned Alaskan sled dogs (2 to 8.5 years old) that were in training for a multiday endurance race.

Procedures—Voided urine samples were collected from 4 groups of dogs (randomly selected from 54 dogs) after no exercise (control group; n = 6 dogs), completion of a 160-km run (group A; 3), completion of a 420-km run (group B; 7), and completion of a 560-km run (group C; 6). Urine cortisol concentrations were determined by use of an immunoassay technique; urine vanillylmandelic acid and homovanillic acid concentrations were measured via high-performance liquid chromatography.

Results—Compared with the control group, urine cortisol concentration in groups A, B, and C was significantly different ($5.33 \times 10^{-4} \pm 2.62 \times 10^{-4}$ µg/dL vs $1.04 \times 10^{-4} \pm 2.31 \times 10^{-5}$ µg/dL, $8.88 \times 10^{-4} \pm 5.49 \times 10^{-4}$ µg/dL, and $6.31 \times 10^{-4} \pm 5.09 \times 10^{-4}$ µg/dL, respectively). Urine homovanillic acid concentration did not differ among the 4 groups. Vanillylmandelic acid was not detected in any urine samples.

Conclusions and Clinical Relevance—Results indicated that prolonged exercise by sled dogs did not affect urine homovanillic acid concentration but did increase urinary cortisol secretion, which is indicative of adrenocortical stimulation. The apparent lack of vanillylmandelic acid in voided urine samples requires further investigation. (*Am J Vet Res* 2007;68:107–111)

In humans, strenuous exercise, especially prolonged exercise such as participating in marathons and triathlons, is associated with increased adrenocortical and adrenomedullary stimulation.[1] It is unknown whether prolonged exercise by well-conditioned sled dogs stimulates adrenergic responses as it does in humans. Adrenergic activation is associated with increases in plasma concentrations of dopamine, cortisol, epinephrine, and norepinephrine as a result of increased adrenal gland secretion. Cortisol and the degradation products of epinephrine, norepinephrine, and dopamine can be measured in the urine as markers of adrenocortical and adrenergic stimulation.[2]

In serum or extracellular fluid, dopamine is degraded to HVA, whereas epinephrine and norepinephrine are metabolized to VMA.[1] Cortisol is excreted unaltered into urine. Plasma cortisol, epinephrine, norepinephrine, and dopamine concentrations can also be measured as indicators of adrenergic stimulation, but numerous psychologic and physiologic processes and technical issues complicate this approach, especially with respect to catecholamines. Measurement of plasma concentrations of these compounds gives insight into conditions at a specific moment in time but does not reflect long-term changes because the compounds are labile and concentrations in blood change rapidly. Additionally, the half-lives of the catecholamines are short, and the peak concentration in the plasma might be undetected even with frequent sample collections. Blood sample collection can be stressful to dogs, thereby increasing catecholamine concentrations in blood and confounding the results. Measurement of degradation products in urine provides an estimate of the amount accumulated since the last time the urinary bladder was emptied. Therefore, the purpose of the study reported here was to determine whether prolonged exercise by conditioned sled dogs affects urine concentrations

Abbreviations

HVA	Homovanillic acid
VMA	Vanillylmandelic acid
DOPA	Dihydroxyphenylalanine

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of HVA, VMA, and cortisol as an assessment of adrenocortical or adrenergic stimulation and adrenal gland response to prolonged exertion. In a previous study,[3] plasma cortisol concentrations increased in sled dogs soon after exercise but urine samples were not examined for markers of catecholamine release. Via measurement of the by-products of adrenergic stimulation in urine, an indication of the adrenal gland status for the duration of the exercise period (rather than a focal time point) is obtained. The effect of blood sample collection is also avoided because urine can be collected as it is voided. Results of analyses of urine for markers of the release of different catecholamines can better characterize the metabolic changes associated with exercise that are responsible for maintaining increased perfusion, blood pressure, and other physical changes necessary for athletic success. It was hypothesized that all of the markers of catecholamine release would increase in response to prolonged exercise.

Materials and Methods

Dogs—The Animal Use and Care Committee of Oklahoma State University approved the study. Of 54 available conditioned Alaskan sled dogs, 24 were used in our study; the owners were aware of and consented to the study procedures. The dogs were randomly selected by the mushers on the basis of fitness level and compliance with urine collection. The dogs were 2 to 8.5 years old; mean body weight was 23.4 ± 2.7 kg. Dogs were fed a low-carbohydrate, high-fat diet and were fit and in training for a multiday endurance race. Training included exercise 4 days a week; dogs ran progressively increasing distances (as far as 80 km [approx 50 miles]). The dogs were unavoidably rested from training for 9 days prior to the start of the study because of adverse environmental conditions (extreme cold).

Sample collection—Voided urine samples were collected from 6 control dogs (which did not participate in the exercise portion of the study), from 3 dogs following completion of a 160-km run (group A), from 7 dogs following completion of a 420-km run (group B), and from 6 dogs following completion of a 560-km run (group C). The groups of dogs were unequal because some dogs did not urinate or urinated before a urine sample could be collected. The dogs ran in teams of 16 and pulled a lightly laden sled and musher over packed snow; the ambient temperatures were –28.9° to –34.4°C (–20° to –30°F). The dogs ran a distance of 70 km during the 5.5-hour period preceding urine sample collection. The distances and times were determined from global positioning satellite data collected by units carried by the musher. The first voided urine sample was collected from the dogs when they stopped running, usually within 5 minutes of the end of the run, by use of a plastic bag attached to a harness. These dogs almost never void urine while running. Within 10 minutes of collection, a 1.8-mL aliquot of each urine sample was acidified with 0.2 mL of 6M hydrochloric acid for analysis of HVA and VMA concentrations. By use of commercially available urine dipsticks,[a] an aliquot of unacidified urine was analyzed prior to freezing for the presence of ketones, glucose, protein, bilirubin, and blood, and pH was determined. Acidified and unmodified urine samples were frozen in liquid nitrogen and shipped frozen at –80°C in appropriate containers from Alaska to Ohio within 24 hours. Samples were stored at –80°C until analyzed. Four urine samples (2 obtained from dogs that ran a distance of 420 km and 2 obtained from dogs that ran a distance of 560 km) were discarded from analysis of catecholamine degradation products because of labeling errors.

Sample analysis—Cortisol concentrations in the urine samples were determined with an automated immunoassay system[b] at The Ohio State University Veterinary Diagnostic Laboratory. Creatinine concentrations in the urine samples were determined by use of an automated chemistry analyzer[c]; this variable was assessed as a correction factor for urine volume to aid in the evaluation of urine cortisol concentration.

Urine VMA and HVA concentrations were determined simultaneously via high-performance liquid chromatography of unextracted samples.[4] High-purity VMA and HVA standards[d] were obtained for use as control samples. Test samples were prepared by adding high-performance liquid chromatography–grade water to the urine sample, filtering that solution through a 0.22-µM nylon membrane filter, and centrifuging the filtrate for 10 minutes. Samples were then transferred to an autosampler, and binary gradient elution was applied (6% mobile phase B for 2.7 minutes, linear gradient to 20% B in 1 minute, and linear gradient to 55% B in 10 minutes). Peak detection, retention time, and response ratio comparison, quantitation, and report generation were performed for each sample. An 8-channel analyzer[e] was used for electrochemical analysis in combination with computer software.[f] Urine samples collected from control dogs were spiked with the metabolites of interest to determine linearity of the assay and minimum levels of detection.[5,6] The methods resulted in linear results within the range of interest (**Table 1**). The lower limit of detection for the assay was 5 µg/mL for VMA and 0.2 µg/mL for HVA. All of the urine samples from the 6 control dogs were used to produce the pooled samples for the validation tests.

The urine samples were analyzed for ketones, glucose, protein, bilirubin, and blood by use of commercial urine dipsticks. All of the samples were negative for ketones, glucose, protein, bilirubin, and blood. The urine specific gravity of all samples was > 1.030, with no significant difference among the groups.

Statistical analysis—Statistical analysis was performed by use of commercial statistical software.[g] The data were examined for normality. If the data were normally distributed, a 1-way ANOVA was performed to compare the 3 exercise groups to each other and to the control group. If the data were not normally distributed, a Kruskal-Wallis 1-way ANOVA for nonparametric data was performed. For purposes of statistical analysis, values of zero were assigned a value of 2.5 mg/L, which was 50% of the lower limit of detection of the assay for VMA. Data were analyzed in 2 ways: data from groups A, B, and C and the control group were individually compared, and data from all exercise groups (ie, A, B,

Table 1—Validation data for the assays used to assess VMA and HVA concentrations in urine samples collected from Alaskan sled dogs.

VMA (μg/mL)			HVA (μg/mL)		
Expected value	Observed value	Observed/expected (%)	Expected value	Observed value	Observed/expected (%)
1,000	989	98.9	1,000	971	97.1
500	507	101	500	513	103
250	285	114	250	323	129
100	70	70	100	4	4
50	34	68	50	23	46
20	24	120	20	5	25
10	14	140	10	40	400
5	13	260	5	12	240

Table 2—Mean ± SD urine HVA, cortisol, and creatinine concentrations and urine cortisol-to-creatinine concentration ratio among Alaskan sled dogs that did not exercise (control group) and those that completed a 160-, 420-, or 560-km run. Values were calculated from samples with detectable compound.

Group (distance run [km])	HVA (μg/mL)	N/T for HVA	Cortisol (μg/dL)	N/T for cortisol	Creatinine (mg/dL)	Cortisol-to-creatinine concentration ratio
Control (0)	10.1 ± 13.7	3/6	14.6 ± 4.71	6/6	139 ± 23	$1.04 \times 10^{-4} \pm 2.31 \times 10^{-5}$
Group A (160)	10.3 ± 13.6	1/3	74.3 ± 52.4*	3/3	97.8 ± 48.0*	$8.88 \times 10^{-4} \pm 5.49 \times 10^{-4}$*
Group B (420)	20.8 ± 40.9	1/5	50.7 ± 41.4*	5/5	81.2 ± 31.6*	$6.31 \times 10^{-4} \pm 5.09 \times 10^{-4}$*
Group C (560)	63.9 ± 54.6	3/4	44.6 ± 25.6*	4/4	85.4 ± 31.4*	$5.33 \times 10^{-4} \pm 2.62 \times 10^{-4}$*
All exercised dogs (groups A–C)	32.5 ± 44.9	5/12	52.8 ± 37.3*	12/12	85.9 ± 32.8*	$6.84 \times 10^{-4} \pm 4.85 \times 10^{-4}$*

*Value significantly ($P < 0.05$) different from that of the control group for this variable.
N/T = Number of samples (1/dog) that yielded positive results for the analyte divided by the total number of samples analyzed from that group of dogs.

and C) were combined into 1 group and compared with control-group data. Values were compared by use of a *t* test if data were normally distributed or a Mann-Whitney rank sum test if data were not normally distributed. The results were considered significant at a value of $P < 0.05$.

Results

All of the urine samples (including the samples collected from the control group) had detectable concentrations of cortisol. Compared with the control-group value, urine cortisol concentration in each of the 3 exercise groups was significantly ($P < 0.05$) different (**Table 2**). Urine creatinine concentration in groups B and C was significantly ($P = 0.004$ and 0.009, respectively) different from that of the control group, whereas urine creatinine concentration in group A was not ($P = 0.086$). Urine creatinine concentration in dogs that were exercised and control dogs was also significantly ($P < 0.05$) different. Compared with the control-group value, the urine cortisol-to-creatinine concentration ratio was significantly ($P < 0.05$) different in each of the 3 exercise groups. Overall, the urine cortisol-to-creatinine concentration ratio differed significantly between exercised and control dogs.

The only metabolite of catecholamine degradation detected in urine samples (including the samples collected from the control group) was HVA. However, HVA concentration in each of the 3 exercise groups was not significantly different from that of the control group; similarly, values in dogs that were exercised and control dogs did not differ. None of the dogs had detectable urine concentrations of VMA, the breakdown product of epinephrine and norepinephrine.

Discussion

Results of the present study indicated that during strenuous exercise, Alaskan sled dogs have sympathetic and adrenomedullary activities that are insufficient to cause detectable spillover of metabolites of epinephrine and norepinephrine into urine. The increased urine concentration of HVA in dogs undergoing exercise, compared with that in unexercised control dogs, is indicative of increased dopamine metabolism during prolonged exercise; the increased urine cortisol concentration in exercised dogs is consistent with increased adrenocortical activity.

Cortisol is a stress hormone that is secreted from the adrenal gland cortex secondary to sympathetic stimulation or an increase in ACTH activity. The half-life of cortisol (60 to 90 minutes in serum) is the longest of the 3 main substances measured in our study. It is not labile and is freely filtered into urine[2]; therefore, it is measured as an intact molecule in urine samples. In the present study, the dogs in all 3 exercise groups had increased urine cortisol concentration, compared with the control-group value. It was expected that cortisol would be detectable in urine from exercised and control dogs because sedentary dogs typically have cortisol in their urine. The difference in urine cortisol concentration between each exercise group and the control group was significant, indicating that exercise is associated with increased release of cortisol and consequently an increase in urine cortisol concentration. This finding is consistent with results of a previous study,[3] which indicated that serum cortisol concentration increased in dogs undergoing exercise, compared with unexercised dogs. An investigation[7] of Alaskan sled dogs that ran shorter distances (sprints of approx 48.3 km [30

miles]) than the dogs of the present study revealed higher serum cortisol concentrations in sprinting dogs, compared with values in unexercised dogs. Urine cortisol-to-creatinine concentration ratios of dogs in the 3 exercise groups of our study were also significantly different, compared with the ratio of dogs in the control group.

Cortisol is released during periods of low glucose availability. The study dogs were fed a low-carbohydrate, high-fat diet. Such a diet might have contributed to the increased urine cortisol concentration because of low availability of simple glucose for utilization, especially during exercise. Blood glucose concentrations remained within the unpublished reference range for sled dogs used by the authors, even though their diet contained low amounts of carbohydrate, because gluconeogenic and glycogenic hormones (eg, cortisol[3]) are released to maintain blood glucose concentration within reference limits.

Urine creatinine concentration was measured as a correction factor for urine volume to aid in the evaluation of urinary cortisol concentration. The decreased urine creatinine concentrations in the exercise groups, compared with the control-group value, indicated that either less creatinine was excreted into the urine or that the urine is more dilute and urine output is increased in exercised dogs.[8] All of the urine samples (including those from the control dogs) had specific gravity > 1.030, and there was no significant difference between the control group and the 3 exercise groups; thus, the dilution of creatinine and other compounds in urine was probably similar among study groups. Therefore, it appears likely that less creatinine was being filtered from the serum into the urine in dogs undergoing exercise than in unexercised control dogs, possibly as a result of a comparative decrease in glomerular filtration rate.

In a previous study[8] of racing sled dogs, urine osmolality after exercise was not different from values before exercise. Creatinine contributes to urine osmolality. If the urine creatinine concentration was significantly different between exercised and unexercised dogs, urine osmolality would also differ. However, such a difference was not detected in the present study; urine osmolality was indirectly assessed via measurement of urine specific gravity, and those values did not differ between control and exercised dogs.

None of the urine samples from any of the dogs (exercised or unexercised) had detectable VMA, the metabolite of norepinephrine and epinephrine. Norepinephrine and epinephrine have plasma half-lives of 10 to 30 seconds, which could be responsible for the lack of epinephrine and norepinephrine urinary metabolites in the dogs of this report.[2] Hormones with such short half-lives are those that are rapidly metabolized; therefore, one would expect to detect their metabolic by-products in urine. Well-trained men have increased urine epinephrine and dopamine concentrations following exercise,[9] whereas plasma VMA but not urine VMA concentrations are increased with exercise.[10] All of the urine samples collected before and after exercise were positive for VMA in both of those reported studies, unlike findings in the dogs of this report. The dogs in the present study could have been excreting VMA at concentrations that were below the limit of detection (5 µg/mL) of the assay used. It would be interesting to measure plasma as well as urine VMA concentrations in exercised dogs to determine whether the former is increased with exercise, as it is in humans.

Homovanillic acid is the major metabolite of dopamine. Dopamine is produced from many sources in the body (including the kidneys) from metabolism of plasma DOPA. Plasma DOPA concentration increases after exercise in dogs and humans.[11,12] Because the dogs in the present study were well trained and underwent frequent exercise, they should have had high concentrations of DOPA in the kidneys, which would enhance production of dopamine and its secretion into the urine.[9] Although the plasma concentration of dopamine is < 25% of that of norepinephrine, urinary dopamine excretion is approximately 10 times as great as that of norepinephrine.[13] This may explain why HVA but not VMA was detected in urine samples from the dogs in the present study (ie, more HVA is excreted into the urine regardless of serum concentration). However, the difference in urine HVA concentration between exercised dogs and control dogs was not significant, which (if correct) is indicative of no change in dopamine metabolism during exercise, changes in renal metabolism of dopamine during exercise, or inadequate statistical power of our analyses. We believe that our finding was most likely attributable to inadequate statistical power because the number of samples in our study was not large.

The findings that the urine samples from the sled dogs in the present study were negative for VMA and that many samples were negative for HVA were unexpected. The lower limit of detection for VMA was 5 µg/mL and HVA was 0.2 µg/mL. In a study[14] involving 9 purebred Beagles, urine VMA concentrations ranged from 0.6 to 15.0 mg/d, although the volumes of urine produced during the study period were not reported. Urine samples were collected once daily from the dogs in our study, and we did not measure the total volume of urine excreted that day for each dog. However, if we estimate that the Beagles in the aforementioned study were typical research animals (weight range, 8.2 to 13.7 kg) and produced approximately 20 mL of urine/kg/d, then sedentary Beagles would have a urine VMA concentration of 3.66 to 54.74 µg/mL, which is above the limit of detection of the assay that we used. This would indicate that the assay used in our study was sufficiently sensitive to detect VMA in the collected urine samples. However, the other study involved clinically normal Beagles, which might not be an appropriate comparison for well-trained Alaskan sled dogs of various ages. It is unlikely that analytical error could account for the failure to detect VMA in urine samples obtained in the present study because the assay was validated and the lower limit of detection was at a physiologically relevant concentration.

If the dopaminergic pathway was preferentially stimulated (thereby increasing plasma concentrations of dopamine but not norepinephrine and epinephrine), then urine concentration of HVA could be greater than that of VMA. The peripheral tissues (ie, the kidneys)

may have preferentially increased the metabolism of dopamine during exercise, resulting in an increase in urine concentration of HVA but not in urine concentrations of epinephrine and norepinephrine metabolites. Although the dopaminergic and adrenergic pathways share some similar effects, they are not interchangeable. In exercising dogs, the dopaminergic pathway may be emphasized by the duration of exercise or previous training. Measurement of epinephrine, norepinephrine, cortisol, and dopamine concentrations in plasma might help determine the cause for the negative findings in the urine samples collected from the Alaskan sled dogs of the present study. If the plasma concentrations of epinephrine and norepinephrine in the study dogs were low, we would conclude that these dogs preferentially used the dopaminergic pathway. If the plasma concentrations of epinephrine and norepinephrine in the study dogs were high, we would consider analytical error or a decrease in metabolism as the most likely causes of urinary metabolites.

Although the Alaskan sled dogs were considered fit, they rested 9 days prior to the start of the experiment because of adverse environmental conditions and the risk of frostbite. The effect of a 9-day rest period on the physical fitness of Alaskan sled dogs has not been determined. The dogs in our study were able to easily complete the required exercise, making it reasonable to speculate that this unanticipated rest period had minimal effect on the outcome of the experiment. Whether the results would have differed had the dogs not had that period of rest is unknown.

The increase in urine cortisol concentration in Alaskan sled dogs participating in endurance exercises, compared with the value in unexercised control dogs, suggests that exercise was associated with adrenocortical stimulation. Further studies are needed to determine why epinephrine and norepinephrine degradation products were not present in urine samples collected from the study dogs and why urine HVA concentration was increased (albeit not significantly) in exercised dogs, compared with unexercised dogs. The presence of HVA but not VMA in urine might indicate that exercised, well-trained dogs preferentially use dopamine rather than epinephrine and norepinephrine to maintain peripheral blood pressure and perfusion. Future studies should include analyses of urine samples for norepinephrine or epinephrine in their unmetabolized forms and plasma samples for epinephrine, norepinephrine, dopamine, and cortisol to determine whether those results are correlated. A larger sample size would also be beneficial to improve the statistical power, thereby enhancing the ability to detect small but biologically relevant differences between groups.

a. Bayer Corp, Elkhart, Ind.
b. Immulite analyzer, Diagnostic Products Corp, Los Angeles, Calif.
c. Hitachi 911 analyzer, Roche, Indianapolis, Ind.
d. Sigma Chemical Co, St Louis, Mo.
e. ESA model 5600A CoulArray detector, ESA Inc, Chelmsford, Mass.
f. CoulArray for Windows software, ESA Inc, Chelmsford, Mass.
g. SigmaStat, version 3.0, SYSTAT Software Inc, Point Richmond, Calif.

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