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Glomerular filtration rate determined by measuring serum clearance of a single dose of inulin and serum symmetric dimethylarginine concentration in clinically normal cheetahs (*Acinonyx jubatus*)

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Objective

To establish a reference interval for glomerular filtration rate (GFR) determined by measuring serum clearance of a single IV dose of inulin in clinically normal cheetahs (*Acinonyx jubatus*) and compare serum symmetric dimethylarginine (SDMA) concentration in cheetahs with GFR.

Animals

33 cheetahs housed at 3 institutions.

Procedures

A single bolus of inulin (3,000 mg/m^2) was administered IV, and 5 serial blood samples were collected and analyzed for serum inulin concentration with the anthrone technique. The GFR was estimated with a modified slope-intercept method for the slow component of the ~~plasma~~1 serum concentration-versus-time curve. Blood urea nitrogen and serum creatinine concentrations were measured in samples obtained immediately prior to inulin administration, and serum SDMA concentration was measured in stored samples.

Results

Mean ± SD measured GFR was 1.58 ± 0.39 mL/min/kg, and the calculated reference interval was 0.84 to 2.37 mL/min/kg. There were significant negative correlations between GFR and serum creatinine concentration (r = –0.499), BUN concentration (r = –0.592), and age (r = –0.463). Serum SDMA concentration was not significantly correlated with GFR (r = 0.385), BUN concentration (r = –0.281), or serum creatinine concentration (r = 0.165).

Conclusions and Clinical Relevance

A reference interval for GFR in clinically normal cheetahs was obtained. Further evaluation of animals with renal disease is needed to determine whether measuring serum clearance of a single IV dose of inulin is a reliable diagnostic test for early detection of renal disease in cheetahs.

ABBREVIATIONS

CI	Confidence interval
CKD	Chronic kidney disease
GFR	Glomerular filtration rate
SDMA	Symmetric dimethylarginine

The cheetah (*Acinonyx jubatus*) is listed as vulnerable in the International Union for the Conservation of Nature's Red List of Threatened Species.[1] As wild populations decrease, captive propagation of cheetahs remains a key element of this species' survival. Yet, captive cheetahs are affected by a number of diseases not observed in their wild counterparts, with CKD being one of the leading causes of death in captive populations.[2] Studies of cheetahs from US zoological parks have found renal lesions, including glomerulosclerosis and nephrosclerosis, in as many as 90% of study populations, with acute kidney injury and CKD cited as the cause of

death in 25% of the animals in 1 study.[3] Most of the US cheetah population is descended from captive populations from the Republic of South Africa, which also demonstrate glomerulosclerosis, although at a lower prevalence.[4] Risk factors for renal disease in cheetahs that have been postulated include reduced genetic diversity, diet, age, chronic stress, and the presence of renal medullary fibrosis.[5–7] In most affected cheetahs, the diagnosis of CKD is not made until the disease is well advanced, at which point treatment is unrewarding.

Most diagnostic methods used to assess renal function include measuring GFR, which represents the volume of fluid filtered by the glomeruli per unit time and is directly related to functional renal mass.[8] Decrements in GFR largely correlate with decrements in other kidney functions.[9] Therefore, GFR is considered to be the best single parameter for assessing renal filtering capacity and overall renal function.[10,11] Although direct measurement of GFR is not possible,[12] it can be indirectly determined by measuring serum concentrations of a filtration marker over time. Clearance of an endogenous filtration marker eases the measurement process by eliminating the need to administer an exogenous marker, and BUN and serum creatinine concentrations are the most commonly used endogenous markers for estimating GFR. However, both BUN and serum creatinine concentrations can be altered by non-renal variables, resulting in a broad range of values in clinically normal animals[13] and limiting their utility for estimating GFR.[14–16] Further, by the time BUN and serum creatinine concentrations are high, at least 75% of nephrons are already non-functional.[17] In contrast, several studies[10,13] have shown that measuring GFR with inulin, an exogenous marker, may identify cats with reduced GFR before BUN or serum creatinine concentration is high.[10,13]

A newer method of assessing renal function is measuring serum SDMA concentration. Symmetric dimethylarginine is a small-molecule byproduct of cellular catabolism recently identified as a novel kidney biomarker in people, cats, and dogs. Like inulin, SDMA is eliminated primarily via glomerular filtration.[18] Although renal excretion has not been proven to be the only means by which SDMA is eliminated from the body, it does appear to represent ≥ 90% of total elimination.[19] Serum SDMA concentration increases in domestic cats with reduced GFR, and in domestic cats, serum SDMA concentration has been shown to be at least as useful as serum creatinine concentration as an indicator of reduced renal function.[18] In at least 1 study,[15] measuring serum SDMA concentration allowed for earlier detection of CKD, compared with measuring serum creatinine concentration. In addition, serum SDMA concentration is more highly correlated with GFR than serum creatinine concentration is, and in contrast to serum creatinine concentration, which decreases with age, serum SDMA concentration increases with age as GFR decreases.[20] A recent study[16] evaluating serum SDMA concentration as a biomarker for early detection of CKD in 7 captive cheetahs found that increases in serum SDMA concentration preceded increases in BUN and serum creatinine concentrations in some of these

animals. That study included animals with known renal lesions and CKD; however, the authors did not compare their results with results of gold standard testing, and the number of animals in the study was limited. Nevertheless, serum SDMA concentration might be useful as an early marker of decreased renal function in this species, and further investigation is warranted.

Measuring the serum clearance of an exogenously administered marker is considered the gold standard method for determining GFR.[21] Although several exogenous substances are available for this purpose, inulin has long been considered to be the ideal marker because it is only cleared by glomerular filtration.[11–13,21–25] Serum clearance of inulin following IV administration of a single bolus has been studied in people[26] and domestic cats,[10,13,27–29] and studies[10,13,29] in domestic cats conclude that measuring inulin clearance is a valuable tool for determining GFR in this species. The present study was developed to evaluate whether inulin clearance could be used to measure GFR in clinically normal cheetahs. Specifically, the purpose of the study reported here was to establish a reference interval for GFR determined by measuring serum clearance of a single IV dose of inulin in clinically normal cheetahs. We also wanted to determine whether serum SDMA, BUN, or serum creatinine concentration was significantly correlated with GFR.

Materials and Methods

Inulin clearance

Serum clearance of inulin following IV administration of a single bolus was measured in 37 cheetahs housed at 3 institutions: the Smithsonian National Zoological Park in Washington, DC (n = 12), the White Oak Conservation Center in Yulee, Fla (16), and the Cheetah Conservation Fund in Otjiwarongo, Namibia (9). There were 17 males and 20 females. Age ranged from 1.2 to 13 years; body weight ranged from 33.5 to 59.5 kg (73.7 to 130.9 lb). Cheetahs owned by the Cheetah Conservation Fund were wild-born animals that had been recently caught, were living in large pens several acres in size, and were fed equine carcasses. Cheetahs owned by the Smithsonian National Zoological Park and White Oak Conservation Center were captive-born, lived in enclosures ranging from 0.25 to 2 acres, and were fed commercial carnivore diets.[a,b]

For measurement of inulin clearance, food was withheld for 12 hours, but animals had free access to water until the morning of the procedure. The cheetahs were anesthetized by IM administration of a combination of xylazine (1.5 mg/kg [0.68 mg/lb]), ketamine (2.2 mg/kg [1 mg/lb]), and midazolam (0.06 mg/kg [0.027 mg/lb]) or a combination of medetomidine (0.03 to 0.035 mg/kg [0.014 to 0.016 mg/lb]), butorphanol (0.2 mg/kg [0.09 mg/lb]), and midazolam (0.12 to 0.15 mg/kg [0.055 to 0.068 mg/lb]). The cheetahs were then intubated, and anesthesia was maintained with isoflurane. Following completion of the procedure, anesthesia was reversed with yohimbine (0.125 mg/kg [0.057 mg/lb], half IV and half IM) in cheetahs that

received the xylazine-ketamine-midazolam combination and with atipamezole (5 mg for every 1 mg of medetomidine, IM), naltrexone (10 mg, IM), and flumazenil (0.2 mg, IM) in cheetahs that received the medetomidine-butorphanol-midazolam combination.

After cheetahs were anesthetized, a physical examination was performed, and an 18-gauge, 1.5-inch catheter was placed in a saphenous or cephalic vein. An initial blood sample was collected in a serum clot activator tube with gel separator for serum biochemical analysis. A single bolus of a 25% solution of inulin[c] (3,000 mg/m^2 body surface area) was then administered IV over 60 seconds through the catheter as described for domestic cats.[10] Standard conversion tables were used to convert body weight to body surface area on the basis of the following equation: body surface area (m^2) = 0.101 × (body weight in kg)$^{2/3}$. The volume of inulin injected ranged from 12.5 to 19.3 mL.

For calculation of GFR, blood samples (3 to 5 mL) were collected into serum clot activator tubes 15, 30, 60, 90, and 120 minutes after inulin administration. In cheetahs in which anesthesia was extended, additional samples were collected 150 and 180 minutes after inulin administration. Samples were centrifuged at XXXX × *g* ~~10,000 RPM2~~ for 5 minutes. The serum was then decanted into 3-mL conical tubes and stored in a refrigerator at < 4° C until all samples had been collected. All samples were then submitted for determination of inulin concentration at the University of Georgia College of Veterinary Medicine. Samples were shipped on dry ice for same-day delivery; serum inulin concentration was measured with the anthrone technique as described.[13]

The study protocol was reviewed and approved by the institutional animal care and use committees at the Smithsonian National Zoological Park, White Oak Conservation Center, and Cheetah Conservation Fund.

Calculation of GFR

The GFR was calculated with a modification of the slope-intercept method for the slow (ie, elimination) component of the serum concentration-versus-time curve.[13,30,31] In short, inulin clearance was determined from the slope and intercept of the serum concentration-versus-time curve determined by linear regression analysis of the final 3 serum inulin concentrations for each cheetah. Clearance was calculated as dose/(slope/intercept).

Serum SDMA concentration

Stored serum samples from 24 of the cheetahs 3 (12 males and 12 females) owned by the Smithsonian National Zoological Park and White Oak Conservation Center were submitted

to Idexx Laboratories, Westbrook, Maine, for determination of serum SDMA concentration. Briefly, SDMA concentration was quantified with a competitive homogeneous immunoassay that incorporated an enzyme-multiplied immunoassay technique.[16,32] This method of measuring serum SDMA concentration has been previously validated in cheetahs.[33]

Statistical analysis

For age, GFR, serum SDMA concentration, BUN concentration, and serum creatinine concentration, descriptive statistics were calculated and histograms were constructed for all cheetahs as a single group and for cheetahs grouped on the basis of sex and facility. The Shapiro-Wilk test was used to determine whether data were normally distributed, and standard normal curves were fitted to histograms. Heterogeneity of variance was examined with the Levene test. General linear models were used to test whether mean values varied by sex or with age. Because of violations for heterogeneity and skewness, serum SDMA concentration was transformed to natural logarithms prior to testing. The Pearson product-moment method was used to test for correlations among variables, and the Fisher z-transformation was used to compare correlation coefficients. All calculations and tests were performed with standard software.[d,e] Values of $P \leq 0.05$ were considered significant.

A reference interval for GFR was determined on the basis of recommendations from the American Society for Veterinary Clinical Pathology[34]; standard software was used.[35] The Horn algorithm with Tukey interquartile fences was used to detect outliers. Because data were found to be normally distributed with the Shapiro-Wilk test, parametric methods were used to establish 90% confidence intervals around the upper and lower reference limits.

Results

No adverse effects associated with IV administration of inulin were reported, and all cheetahs recovered well from anesthesia and returned to their routines shortly after the procedure. Three cheetahs were removed from the study because of insufficient samples, and 1 was removed because of high BUN and serum creatinine concentrations, compared with reference intervals.[36] Thus, GFR was calculated for 33 cheetahs (16 males and 17 females). Values for GFR were approximately normally distributed, and no outliers were identified **(Figure 1)**. The observed mean ± SD GFR was 1.58 ± 0.39 mL/min/kg **(Table 1)**. The calculated reference interval was 0.84 to 2.37 mL/min/kg, with 90% CIs of 0.62 to 1.00 mL/min/kg for the lower reference limit and 2.15 to 2.53 mL/min/kg for the upper reference limit.

There were significant negative correlations between GFR and serum creatinine concentration ($r = –0.499$; $P = 0.003$), BUN concentration ($r = –0.592$; $P = 0.002$), and age ($r = –0.463$; $P = 0.007$). Mean GFR did not differ significantly ($P = 0.303$) between male and female cheetahs,

but mean serum creatinine concentration did (P = 0.013), with females having a higher mean value than males. The GFR decreased and the BUN concentration and serum creatinine concentration increased as age increased.

Serum SDMA concentration was not significantly correlated with GFR (r = 0.385; P = 0.063), serum creatinine concentration (r = 0.165; P = 0.442), or BUN concentration (r = –0.281; P = 0.180) and did not differ significantly (P = XXXX)4 between male and female cheetahs. In addition, serum SDMA concentration did not significantly (P = XXXX)4 increase with age. The Fisher z-transformation was used to compare the SDMA-versus-GFR and SDMA-versus-serum creatinine concentration correlation coefficients. There was no significant (P = 0.401) difference between these 2 coefficients.

Because age was significantly correlated with GFR, BUN concentration, and serum creatinine concentration, partial correlations were computed with age held constant, and GFR was still significantly correlated with BUN concentration (r = –0.348; P = 0.05) and with serum creatinine concentration (r = –0.399; P = 0.024).

Discussion

In the present study, a reference interval for GFR determined by measuring serum clearance of a single IV dose of inulin in clinically normal cheetahs (0.84 to 2.37 mL/min/kg) was calculated. A previous study[37] used urinary clearance of creatinine to estimate GFR in 12 cheetahs and found that this method was consistent with using urinary clearance of inulin as a measure of GFR in this species. Our study distinguished itself from that previous study in that we used a larger population of cheetahs located in 3 facilities, used only a single IV injection of inulin, and calculated GFR on the basis of serum clearance rather than urinary clearance. Nevertheless, our results for calculation of GFR were consistent with values for GFR reported in that previous study[37] (mean ± SD, 1.59 ± 0.17 mL/min/kg body weight). To our knowledge, the present study represented the first time that values for GFR determined on the basis of serum clearance following a single IV injection of inulin in cheetahs have been reported. The reported values will likely be useful for evaluating renal function in this species and potentially could be used to detect renal disease before less sensitive markers, such as BUN and serum creatinine concentrations, change. However, further evaluation in cheetahs with abnormal renal function is needed before concluding that measuring serum clearance of a single IV dose of inulin could be a reliable diagnostic test for the early detection of renal disease in cheetahs.

The primary limitation of our study was that only cheetahs with no evidence of CKD, as determined on the basis of results of a physical examination and serum biochemical testing,

were included. In addition, we could not determine whether any external factors such as dehydration secondary to fasting had an effect on measured GFR values. It is also possible that variation in dietary protein concentration among study participants could have altered baseline BUN and serum creatinine concentrations and measured GFR. Finally, the effect of anesthesia on GFR was not assessed. It is possible that the effects of anesthesia on cardiac output and blood pressure could influence GFR, and the 2 anesthetic protocols used in this study could have had differential effects on GFR. The effects of various anesthetic drugs on GFR in dogs have been evaluated, and medetomidine has been found to decrease GFR.[38] However, similar alterations in measured GFR were not observed in cats when the effects of sedation on GFR were evaluated with 3 sedation protocols, including 1 with medetomidine.[39] Intrinsic autoregulatory mechanisms often maintain GFR despite decreases in systemic blood pressure or renal blood flow.[40] Additional studies are warranted to determine the possible effects of anesthesia on GFR in non-domestic felids generally and cheetahs specifically.

Although not part of the original study design, we saw value in measuring serum SDMA concentration in cheetahs for which stored samples were available. A 2006 meta-analysis[41] could not determine whether SDMA fulfils all the necessary criteria as a marker for GFR in humans, but the authors thought that it held promise because serum concentrations correlated with values for other established methods of GFR determination and measures of renal function.

In the present study, serum SDMA concentration was not correlated with either BUN or serum creatinine concentration or with GFR. We acknowledge, however, that serum SDMA concentration was measured in only a subset (n = 24) of the samples. Waugh et al[33] retrospectively evaluated 92 stored serum samples from 11 cheetahs with renal disease and found that serum SDMA and creatinine concentrations were highly correlated in individual cheetahs for which serial samples were available.[41] Lamglait and Vandenbunder-Beltrame[16] also retrospectively evaluated a population of cheetahs that had died of CKD and found that among 5 of 7 animals for which banked serum and plasma samples (n = 58) were available had marked increases in serum SDMA concentration before serum creatinine and BUN concentrations increased. However, serum SDMA concentration was not compared with a gold-standard test such as inulin clearance in either of these studies. One explanation for the lack of correlation in the present study could be that serum SDMA concentration correlates better with GFR than BUN or serum creatinine concentration does when animals with a greater range of renal function, especially decreased renal function, are included in the analysis.

The assay used to determine serum SDMA concentration that was used in the present study has been validated for dogs and cats[32] and cheetahs[33]; however, a reference interval for cheetahs

has not been established, and it is possible that the reference interval for cheetahs is different from that for domestic cats. Because SDMA is an endogenous substance, its use as a marker of renal function does not require exogenous administration, making it appealing for application in clinical zoo animal and wildlife medicine. Given this and the previous reports of serum SDMA concentration in cheetahs with compromised renal function, we strongly believe that further investigation of its usefulness in non-domestic felid species is warranted.

Haller et al[10] assessed whether GFR could be estimated in domestic cats by measuring serum inulin concentration at a single timepoint 180 minutes after IV injection of inulin (3,000 mg/m^2). They reported excellent sensitivity and good specificity for this technique and concluded that it was a simple, easy, and cheap method of obtaining more information about renal function in cats than measurement of serum creatinine concentration. The reference interval developed in the present study for GFR in clinically normal cheetahs could potentially help in the earlier diagnosis of renal disease in this species. Although multiple blood samples were collected in the present study to help establish the reference interval, it is possible that a more practical method involving collection of fewer blood samples could be developed.

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Footnotes

a. Zoo Carnivore Diet, Natural Balance Pet Foods Inc, Burbank, Calif.
b. Milliken Meat, Milliken Meat Products Ltd, Markham, ON, Canada.
c. Inutest (25% solution in sterile water), Fresenius Kabi Austria GmbH, Linz, Austria.
d. SPSS version 24, IBM Corp, Armonk, NY.
e. Mathcad, PTC, Boston, Mass.

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Figure 1—Histogram of GFR determined by measuring serum clearance of a single IV dose of inulin in 33 clinically normal cheetahs (*Acinonyx jubatus*). The solid line represents the fitted standard normal curve (mean, 1.58 mL/min/kg; SD, 0.388 mL/min/kg).

8~~**Table 1**—Anesthetic protocols used in each facility for inulin administration~~

Table 1—Descriptive statistics for age, GFR (determined by measuring serum clearance of a single IV dose of inulin), serum creatinine concentration, BUN concentration, and serum SDMA concentration in 33 clinically normal cheetahs (*Acinonyx jubatus*).

Variable	Mean	Median	SD	Range	95% CI for mean
Age (y)	4.6	2.8	3.5	1.2–13	3.4–5.9
GFR (mL/min/kg)	1.58	1.48	0.39	0.98–2.68	1.45–1.72
Creatinine (mg/dL)	2.46	2.30	0.42	1.9–3.4	2.3–2.6
BUN (mg/dL)	38.8	37.9	6.7	27–55	37–41
SDMA (µg/dL)*	16.7	16.0	4.7	11–35	15–19

*Measured in stored samples for 24 of the 33 cheetahs.