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Plasma exogenous creatinine clearance in clinically healthy cats: Comparison with urinary exogenous creatinine clearance, tentative reference intervals and indexation to bodyweight

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ABSTRACT

Glomerular filtration rate (GFR) is considered to be the best indicator of overall kidney function. The major objectives of this study were to compare plasma exogenous creatinine clearance (PECC) with a reference method, to establish reference intervals (RIs) for PECC and to assess the effects of indexation of GFR to bodyweight (BW) in cats. PECC was compared with urinary clearance of exogenous creatinine (UECC) in six clinically healthy domestic shorthair cats (experiment 1). Tentative RIs were determined according to current guidelines and the effects of indexation to BW and of covariables on GFR were assessed in 43 clinically healthy cats of various breeds (experiment 2).

PECC was 15% higher than UECC ($P < 0.01$), but the two estimates were strongly correlated ($r^2 = 0.97$, $P = 0.001$). RIs for PECC were 6.4–21.3 mL/min or 1.2–4.9 mL/min/kg. The absolute (i.e. non-indexed) GFR value was not dependent on BW. Thus, indexation of GFR to BW in cats would not standardize the GFR value, but could introduce bias in clinical interpretation. Significant effects of breed, plasma protein concentration and plasma albumin concentration on GFR were demonstrated. Plasma concentrations of urea and creatinine, when assessed separately, were also weakly correlated with GFR in healthy cats. These combined findings contribute to a better understanding of renal function assessment in cats.

Introduction

Chronic kidney disease (CKD) is a common health problem that causes considerable morbidity and mortality in cats (Lees, 2004). Glomerular filtration rate (GFR) is considered the best indicator of overall renal function (Lefebvre, 2011). In clinical settings, the identification of declining kidney function currently relies on a comparison of the plasma/serum creatinine concentration with the upper limit of a reference interval (RI). However, plasma creatinine concentration (P-creatinine) is known to be an indirect and insensitive indicator of GFR (DiBartola, 2010).

There are two main reasons for the insensitivity of P-creatinine: firstly, it is influenced by factors other than GFR, notably the endogenous creatinine production rate, which is related to muscle mass (Le Garreres et al., 2007), and, secondly, the relationship between P-creatinine and GFR is curvilinear in cats (Miyamoto, 2001a). In practical terms, P-creatinine remains relatively constant as GFR declines in the early stages of CKD, which explains its poor sensitivity as a screening tool (Heiene and Lefebvre, 2006). However, early identification of CKD could be beneficial, as therapeutic intervention is likely to halt or slow disease progression more efficiently when implemented early in the course of renal disease (Lees, 2004). Practical and accurate methods for measuring GFR would facilitate the early detection of CKD.

GFR can be calculated from the urinary or plasma clearance of appropriate markers. Exogenous creatinine and iohexol have been extensively used as markers in feline medicine (Becker et al., 2000; Miyamoto, 2001a; Le Garreres et al., 2007; van Hoek et al., 2008, 2009a; Goodman et al., 2009; Reynolds et al., 2013). Plasma clearance of iohexol is suitable for GFR measurement in cats (Brown et al., 1996a; Miyamoto, 2001b) and the pharmaceutical formulations approved for use in humans can be used. However, high-performance liquid chromatography, an analytical method not routinely available, is required for the assay. Moreover, a potential adverse effect in humans is contrast-induced nephropathy (Solomon and Dumouchel, 2006), although, despite being studied, this clinical

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complication has, to the best of our knowledge, never been reported in dogs and cats. Exogenous creatinine has been considered an appropriate marker for both urinary and plasma clearance in dogs and cats (Lefebvre, 2011), and assays are readily available, inexpensive and require very small specimen volumes. Exogenous creatinine is safe when administered IV in cats (Heiene et al., 2009), but a medical grade formulation is not commercially available.

Plasma clearances of iohexol or exogenous creatinine in cats have not been directly compared with urinary clearance of inulin, the reference standard for measuring GFR (Von Hendy-Willson and Pressler, 2011). Moreover, plasma clearance methods have been rarely validated against urinary clearance of inulin (McClellan et al., 2006) and urinary clearance of exogenous creatinine (UECC) has mostly been used as the reference method (Rogers et al., 1991; Brown et al., 1996a, 1996b; Miyamoto, 2001b). Comparison of UECC with the reference standard has been documented and UECC has been shown to provide an accurate measure of GFR in cats (Finco and Barsanti, 1982; Brown et al., 1996b), making it an accepted method for the measurement of GFR in this species (Rogers et al., 1991; Brown et al., 1996a, 1996b; Miyamoto, 2001a, 2001b).

In studies performed to establish the validity of iohexol for GFR measurement, plasma iohexol clearance was compared with UECC (Brown et al., 1996a; Miyamoto, 2001b). Plasma clearance of exogenous creatinine (PECC) has been used in cats (Le Garreres et al., 2007; van Hoek et al., 2007, 2008, 2009a, 2009b; Heiene et al., 2009; Reynolds et al., 2013). However, it has not been compared with any urinary clearance method to date. Only correlations with alternative methods, i.e. plasma clearance of inulin (Miyamoto, 1998) or iohexol (Le Garreres et al., 2007; van Hoek et al., 2007; Heiene et al., 2009) have been reported.

Importantly, the conclusions of many studies on methods for GFR measurement in cats are intrinsically flawed because they are based on comparisons between different methods (Russo et al., 1986; Miyamoto, 1998; Reichle et al., 2002; Le Garreres et al., 2007; van Hoek et al., 2007; Heiene et al., 2009; Sox et al., 2010; Chang et al., 2011; Granger et al., 2012; Schmidt et al., 2012; Katayama et al., 2013) and/or comparisons of results obtained from methods based on different pharmacokinetic models (Russo et al., 1986; Miyamoto, 1998) and/or sampling strategies that are limited and unvalidated (Vandermeulen et al., 2008, 2010; Heiene et al., 2009; Miyagawa et al., 2010; Finch et al., 2011; Katayama et al., 2013).

Whatever the method used, GFR values are most commonly indexed to bodyweight (BW), i.e. expressed in mL/min/kg, in an attempt to minimize any variation related to body size. However, normalization to body size has not been standardized in small animals (Goy-Thollot et al., 2006) and the ideal indexation method remains unknown (Von Hendy-Willson and Pressler, 2011). Three ways to scale GFR have been proposed, based on bodyweight (BW), body surface area (BSA) or extracellular fluid volume (ECFV). In cats, the data available on GFR standardization are limited and conflicting (Goy-Thollot et al., 2006; Heiene et al., 2009). GFR can be influenced by body size, age, gender or breed (Von Hendy-Willson and Pressler, 2011). The effects of age in cats are also a point of debate (van Hoek et al., 2007; Heiene et al., 2009).

The first objective of the present study was to compare the PECC test (PECCT) with the UECC, a widely accepted method for GFR measurement, in cats (Brown et al., 1996b). In addition, the use of creatinine to measure concomitant plasma and urinary clearance was thought to be of value for better understanding of the kinetics of this marker. The second objective was to define a tentative RI for GFR measured by PECCT according to current guidelines of the Clinical and Laboratory Standards Institute (CLSI; CLSI, 2000, 2008). In addition, the effects of indexation to bodyweight and of co-variables on GFR were assessed in the same population of healthy cats.

Table 1
Study design for experiment 1.

	Period 1	Period 2
Sequence 1	SHAM	ECCT
Sequence 2	ECCT	SHAM

SHAM, sham procedure for 24 h baseline endogenous creatinine excretion measurement; ECCT, combined exogenous creatinine plasma/urinary clearance test.

Materials and methods

Experiment 1: Comparison of PECC and UECC

Cats

Six clinically healthy female domestic shorthair (DSH) cats from a research colony, weighing 3.9 ± 0.5 (3.2–4.5) kg and aged 8.7 ± 1.8 (6.5–10.5) years, were used. They were declared healthy based on medical history, clinical examination, plasma biochemistry results and a post-study follow-up to ensure that they had remained clinically healthy for 3 months after testing. The study protocol was approved by the Regional Committee for Ethics in Animal Experiments (CEEA.2012.150).

Study design

A prerequisite for the calculation of exogenous urinary clearance was to measure individual 24 h baseline endogenous creatinine urinary excretion. The creatinine excreted over 24 h after exogenous creatinine administration is of both endogenous and exogenous origin. Therefore, the amount of exogenous creatinine excreted in 24 h, which is required to calculate its urinary clearance, can only be derived by subtracting the fraction of endogenous origin from the total amount of creatinine excreted.

A 24 h urine collection was performed twice from each cat, at a 1-week interval. One period was for the combined exogenous creatinine plasma/urinary clearance test (ECCT), while the other was for a sham procedure (i.e. without exogenous creatinine administration and repeated blood sampling) to measure individual 24 h baseline endogenous creatinine excretion (SHAM). All six cats were subjected to both procedures in a crossover design according to two distinct sequences (Table 1). One sequence was randomly attributed to each cat. For randomization, the cats were ranked according to age and BW and paired. The first cat in each pair was randomly assigned (coin flip) to one sequence and the second cat was assigned to the other sequence.

Procedures – SHAM

Cats were fasted for 12 h before testing but had free access to water. On the day of testing, all cats were weighed, an IV catheter was inserted in the cephalic vein and secured. Ten milligrams of ketamine chlorhydrate (Clorketam, Vetoquinol) and 0.5 mg of diazepam (Valium, Roche) mixed in the same syringe were injected via the catheter for short-term chemical restraint, as previously described (Reynolds et al., 2012). The urethra was catheterized with a sterile urinary catheter (Buster cat catheter, Kruuse) and the bladder was completely emptied. The time at the end of bladder emptying was recorded.

Collected urine was discarded and the cat was then placed in an individual cage equipped with a urine collection system connected to an empty vial of known weight. Thirty minutes later, free access to water was allowed. Eight hours later, the cats were individually offered access to their usual daily food intake for 1 h and were then fasted again. Twenty-four hours later, the same procedure was repeated for bladder emptying.

Urine collected from the bladder was mixed in the same vial with all the urine collected from the cage and specific gravity was measured with a clinical refractometer (Digital Urine S.G. Refractometer UG-1, Atago). The vial was weighed. The empty bladder was then flushed (through the urinary catheter) with 10 mL of sterile 0.9% NaCl. Bladder flushing was repeated three times. The entire contents of each flush were placed in a separate empty vial of known weight and weighed. The time at the end of bladder flushing was recorded. The tray of the cage was rinsed three times with 100 mL of distilled water (for each rinsing) at 30 min intervals. The water from each rinse was collected in a separate empty vial of known weight connected to the urine collection system of the cage. The three vials containing rinsing water were weighed.

Procedures – ECCT

Creatinine solution (80 mg/mL) was prepared the day before testing by dissolving anhydrous creatinine (Sigma) in distilled water. The solution was sterilized by filtration through a 0.2 μm filter. The exact individual dose was determined from the syringe weight. The same procedure as for SHAM was followed, but an IV bolus of exogenous creatinine solution (20 mg/kg) was administered through the already placed cephalic catheter, immediately after initial bladder emptying. In addition, blood for PECCT was collected in lithium heparin tubes from a cephalic vein, as described elsewhere (Reynolds et al., 2007), before and at 5 and 30 min, and 1, 2, 3, 5 and 8 h after creatinine administration.