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Determination of Extracellular Fluid Volume in Healthy and Azotemic Cats

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Background: Methods for determining extracellular fluid volume (ECFV) are important clinically for cats. Bromide dilution has been studied in cats to estimate ECFV. Markers of GFR also distribute in ECFV and can be used for its measurement.

Hypothesis/Objectives: The primary objective was to develop a method of determining ECFV from iohexol clearance in cats and evaluate agreement with that determined using bromide dilution. Additional objectives were to compare ECFV between azotemic and nonazotemic cats and evaluate appropriate methods of standardizing ECFV.

Animals: Client-owned cats with varying renal function.

Methods: Validation of ECFV determined from slope-intercept iohexol clearance was performed in 18 healthy nonazotemic cats. ECFV was then determined using the validated method and bromide dilution and agreement assessed. Appropriateness of standardization to body weight (BW) and body surface area (BSA) was evaluated.

Results: Extracellular fluid volume determined from slope-intercept iohexol clearance and bromide dilution was 0.84 ± 0.32 L and 0.85 ± 0.19 L (mean ± SD), respectively. There were wide limits of agreement between the methods (−0.58 to 0.54 L) and therefore, agreement was considered to be poor. ECFV did not differ significantly between azotemic and nonazotemic cats (P = .177). BSA was found to be the best method for standardizing ECFV measurement in cats.

Conclusions and Clinical Importance: This study developed a method for determining ECFV from slope-intercept iohexol clearance which provides simultaneous assessment of renal function and an estimate of ECFV. ECFV does not differ between azotemic and nonazotemic cats, which suggests fluid volume loss or overload is not an important clinical feature in cats with mild chronic kidney disease.

Key words: Bromide; Glomerular filtration rate; Iohexol; Kidney disease; Renal.

Clinically useful methods of measuring extracellular fluid volume (ECFV) are lacking in cats. Development of such methods could lead to enhanced understanding of pathophysiological processes in which there is an alteration in ECFV and for monitoring fluid status. Simultaneous measurement of ECFV and glomerular filtration rate (GFR) could be important in evaluating cats with renal disease including acute kidney injury or chronic kidney disease (CKD) and also secondary complications such as hypertension.

Dilution methods for determining ECFV involve administration of tracers such as saccharides (inulin, or mannitol), thiocyanate, sulfate, chloride, or bromide. These methods are based on Fick's principle which states that the volume of a fluid space may be calculated after administration of a marker, if the concentration of marker in fluid after complete mixing is known. This requires, that the marker distributes and equilibrates within a predictable time period throughout its volume of distribution (ECFV), does not influence fluid fluxes across membranes and that its clearance or metabolism is negligible. Bromide falls close to meeting these requirements, is considered the most accurate and most commonly studied tracer in humans.[1] However, because of differences in molecule size and physiochemical properties, all tracers have slightly different behavior across biological membranes and so their distribution space will naturally display some variation.[2] The apparent volume of distribution of most substances (including plasma clearance markers used to determine GFR) is generally not an anatomically defined fluid space. Most GFR markers distribute within a volume close to ECFV while the distribution volume of creatinine is considered closer to that of total body water.[3,4] Iohexol is a satisfactory plasma clearance marker for estimating GFR

Abbreviations:

^{51}Cr-EDTA	chromium-51-ethylenediaminetetraaceticacid
^{99}Tc-DTPA	technitium-99 m-diethylenetriaminepentacetic acid
AKI	acute kidney injury
AUC	area under the plasma concentration vs. time curve
BSA	body surface area
BW	body weight
CKD	chronic kidney disease
Cl	clearance
ECFV	extracellular fluid volume
GFR	glomerular filtration rate
HPLC	high performance liquid chromatography
ICP-MS	inductively coupled mass spectrometry
IRIS	International Renal Interest Society
MRT	mean residence time
PCV	packed cell volume
TBW	total body water
Vd	volume of distribution

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with a distribution volume in various species approximating ECFV.[5–7] Determination of clearance using this marker could provide simultaneous measurement of ECFV. The distribution volume of bromide is not necessarily true ECFV anymore than the distribution volumes of other tracers. The clinical utility of being able to assess GFR and ECFV concomitantly makes measurement of the distribution volume of iohexol more attractive.

Methods

Client-owned cats with varying levels of renal function were included in the study. Renal azotemia was defined as a plasma creatinine concentration above the laboratory reference interval (>2.0 mg/dL) in association with reduced urine concentrating ability (urine specific gravity <1.035). Cats with evidence of concurrent medical disease were excluded. There were a total of 89 cats in which ECFV was determined from iohexol clearance. Of these 89 cats, 66 cats also had concurrent measurement of ECFV using bromide dilution. Informed consent was obtained from the owners and the study was conducted with approval from the Royal Veterinary College's ethics and welfare committee. Food was withheld for 12 hours before the measurements and water was withheld during the measurements.

Determination of ECFV Using Bromide Dilution

A 1 mL blood sample was collected at baseline before administration of bromide and transferred into a serum tube. This sample was used to determine naturally occurring concentrations of bromide in blood. A sterile nonpyrogenic 30% solution of NaBr was administered at a dose of 30 mg/kg via an intravenous catheter placed in the cephalic vein. Exact dose of tracer administered was calculated from the weight of the syringe before and after administration. The syringe was weighed using digital laboratory scales[a] which were calibrated regularly. An equilibration period of 2 hours was allowed before collection of the postdose sample via jugular venepuncture into a serum tube. This plateau phase of equilibration has previously been validated in cats.[8,9] Serum was harvested and transferred to storage at −80°C before analysis. Sample analysis of bromide was performed at an external commercial laboratory[b] using inductively coupled mass spectrometry (ICP-MS).

The dilution space of bromide was calculated using the following equation:

$$ECFV_{Bromide} = \frac{Br_{dose}}{Br_p - Br_b} \times 0.95 \times 0.90$$

where Br_{dose} is dose of bromide administered (mmol), Br_p is postdose bromide sample (mmol/L), Br_b is baseline bromide sample (mmol/L), 0.95 is the Donnan correction and 0.9 is correction for intracellular (mainly erythrocyte) penetration. $ECFV_{Bromide}$ was expressed in L.

Volume of distribution is calculated from the full plasma clearance curve using the following equation:

$$Vd_{Iohexol} = MRT \times Cl$$

where MRT is the mean residence time in its distribution volume in minutes, Cl is the plasma clearance rate of iohexol in mL/min and $Vd_{Iohexol}$ is assumed to equate to ECFV. As iohexol clearance equates to GFR the equation can be rearranged:

$$GFR/ECFV = MRT^{-1}$$

Slope-intercept clearance can be determined from 3 blood samples in cats with a correction applied for the one-compartment assumption.[10] The single elimination exponent generated from the slope-intercept clearance curve (β) slightly underestimates GFR/ECFV because of the one-compartment assumption.[11,12] A correction factor can be applied to obtain GFR/ECFV from β and has been validated for humans[13] but not cats.

Studies measuring ECFV in cats have focused on methods to obtain data and not how the data are expressed. A consensus has not been reached for humans and volumes are generally reported in liters (L). This approach is unsatisfactory as a patient with larger body size will have corresponding larger fluid volumes. Suggested methods of standardizing measurements include body weight (BW) and body surface area (BSA).

The objectives of this study were 4-fold:

1 to validate a correction factor for the one-compartment assumption to determine GFR/ECFV from β
2 to assess agreement between ECFV calculated from GFR/ECFV with that determined using bromide dilution
3 to compare measurements between azotemic and nonazotemic cats
4 to report an appropriate method for standardizing ECFV measurements in cats.

Determination of ECFV Using Iohexol Plasma Clearance and GFR/ECFV

Multisample and slope-intercept iohexol clearance was measured using a previously described method.[10] Briefly, a bolus dose of iohexol (Omnipaque [647 mg/mL; 300 mg of iodine/mL]) was administered IV (1 mL/kg). For the multisample method, blood samples were collected at 5, 15, 30, 60, 120, 180, 240, and 360 minutes. For the slope-intercept method blood samples which were collected at 120, 180, and 240 minutes were used. Iohexol concentrations were determined at an external commercial laboratory using a HPLC method.[c] Clearance was determined as dose/AUC where AUC is area under the plasma concentration versus time curve. The AUC for the multisample method was determined using a two-compartment model. The AUC for the slope-intercept method was determined using a one-compartment model. A previously validated cat specific correction formula for slope-intercept clearance was applied to correct for the one-compartment assumption.[10]

GFR/ECFV was determined from the reciprocal of MRT of iohexol from multisample clearance using the following equation:

$$\text{Multisample GFR/ECFV} = \frac{A/\alpha + B/\beta}{A/\alpha^2 + B/\beta^2}$$

where A is the iohexol concentration at the zero time intercept of the first (distribution) exponential, α is the slope of this exponential, B is the iohexol concentration at the zero time intercept of the second (elimination) exponential and β is the slope of this exponential.

GFR/ECFV was determined from slope-intercept clearance using the following equation:

$$\text{Slope-intercept GFR/ECFV} = \beta$$

(if ECFV is in mL, then β is min^{-1})

The cat specific correction factor for slope-intercept GFR/ECFV was obtained by regressing β (obtained from slope-intercept clearance) against the reciprocal of MRT (determined from multisample clearance) and exploring linear, quadratic and cubic models.

Forcing the regression through the origin ensured a constant was not generated in the regression equation. In addition, GFR/ECFV corrected using the feline correction formula was compared with a human correction formula for ^{51}Cr-EDTA[13] and iohexol[14] and a canine correction formula for ^{99m}Tc-DTPA[15] (See Table 1).

Extracellular fluid volume determined from slope-intercept iohexol clearance ($ECFV_{Iohexol}$) was calculated using the following equation:

$$ECFV_{Iohexol} = GFR/(GFR/ECFV)$$

where GFR is determined from corrected slope-intercept clearance and GFR/ECFV is determined from corrected slope-intercept GFR/ECFV as described above. Data derived for ECFV using the equation above were multiplied by BW and divided by 1,000 to obtain results in L.

Standardization of ECFV Measurements

Measurements of $ECFV_{Bromide}$ and $ECFV_{Iohexol}$ were standardized using BW and expressed as L/kg and BSA expressed as L/m^2.

BSA was calculated according to the equation[16]:

$$BSA = K \times BW^a$$

where K is the shape constant (0.1 in cats) and a is the mass exponent (0.66 in cats).[17]

To assess appropriateness of standardization of ECFV, the standardization parameter (BW or BSA), should correlate with unscaled ECFV. However, when the parameter is correlated with ECFV standardized to the parameter (ECFV/kg or ECFV/m^2), then the correlation should not be significant. This was evaluated by performing regression analysis of ECFV/kg on BW or BSA and determining the coefficient of determination (R^2).

Statistical Methods

The data were assessed for normality by visual inspection and by performing the Kolmogorov-Smirnov test. As the data met the assumptions of a Gaussian distribution, parametric testing was used. ECFV was compared between azotemic and nonazotemic cats using the *t*-test. Correlations were explored using Pearson's correlation coefficient. Relationships were evaluated by performing linear regression analysis and determining the coefficient of determination (R^2). Agreement was assessed by plotting the difference of two measurements against their averages (Bland-Altman plots).[18] Bias was defined as the mean difference between two measurements and the absolute limits of agreement were defined as mean difference ± 2SD. Results are presented as mean ± SD except for plasma creatinine concentration, which is presented as median (range). Significance was set at $P < .05$.

Table 1. Formulae for correction of GFR/ECFV using the markers ^{51}Cr-EDTA[13] and iohexol[14] in human patients and ^{99m}Tc-DTPA[15] in dogs. The correction formula for human patients using ^{51}Cr-EDTA was derived through the same method described in this study. The formula for human patients using iohexol and for dogs using the ^{99m}Tc-DTPA was derived from the second-order polynomial relationship of GFR/ECFV determined from 6 sample GFR and slope-intercept GFR.

Species	Marker	Formula
Human	^{51}Cr-EDTA	$\beta + (15.4 \times \beta^2)$
Human	Iohexol	$(1.0526 \times \beta) + (0.0052 \times \beta^2)$
Dog	^{99m}Tc-DTPA	$-0.326 + (1.146 \times \beta) + (0.0020 \times \beta^2)$

Results

Determination of ECFV Using Bromide Dilution

A total of 66 cats had ECFV determined using bromide dilution (55 nonazotemic, 11 azotemic). Descriptive data of cats included in the study are presented in Table 2. There was no significant difference in BW between azotemic and nonazotemic cats ($P = .925$). Azotemic cats were significantly older than the nonazotemic cats ($P = .034$). No adverse clinical signs were reported in any cats during the study period. Mean ± SD $ECFV_{Bromide}$ and $ECFV_{Bromide}$/BSA are presented in Table 3. $ECFV_{Bromide}$ was not significantly different between azotemic and nonazotemic cats either, when standardized to body size or unstandardized ($P > .05$).

Determination of ECFV Using Iohexol Plasma Clearance and GFR/ECFV

Development of the cat specific correction formula for GFR/ECFV was performed in 18 healthy nonazotemic cats. A linear regression model provided the best fit to the relationship between multisample GFR/ECFV and slope-intercept GFR/ECFV ($R^2 = 0.90$, $P < .001$; see Fig 1).

The derived cat correction equation for slope-intercept GFR/ECFV was:

$$GFR/ECFV = 1.027 \times \beta$$

Slope-intercept GFR/ECFV corrected using this feline correction formula showed excellent agreement with multisample GFR/ECFV (see Fig 2). Maximum difference between the two methods was 0.0013 min^{-1}, which was approximately 15% of mean GFR/ECFV for the group of cats.

Eighty-nine measurements of corrected GFR/ECFV were determined from slope-intercept iohexol clearance (73 nonazotemic and 16 azotemic cats). Descriptive data of the cats included in the study are presented in Table 2. There was no significant difference in BW ($P = .221$) and age ($P = .157$) between azotemic and nonazotemic cats. Mean ± SD GFR/ECFV corrected using the feline correction formula, validated human correction formula,[13] human correction formula for iohexol[14] and canine correction formula[15] was 0.0084 ± 0.0025, 0.0082 ± 0.0025, 0.0086 ± 0.0026, and −0.32 ± 0.003 min^{-1} respectively. The feline and human correction formulae all appeared to be appropriate for correcting slope-intercept GFR/ECFV in cats however, the dog formula underestimated GFR/ECFV by approximately 0.32 min^{-1}.

Extracellular fluid volume determined from slope-intercept GFR/ECFV corrected using the feline correc-

Table 2. Descriptive data of cats included in this study. $ECFV_{Bromide}$ is extracellular fluid volume determined using bromide dilution. $ECFV_{Iohexol}$ is extracellular fluid volume determined from corrected slope-intercept GFR/ECFV using the filtration marker iohexol.

	$ECFV_{Bromide}$		$ECFV_{Iohexol}$	
	Nonazotemic Cats (n = 55)	Azotemic Cats (n = 11)	Nonazotemic Cats (n = 73)	Azotemic Cats (n = 16)
Age (years)	12.5 (3.0–19.9)	15.4 (10.4–18.7)	12.5 (3.0–19.9)	13.7 (10.4–18.7)
Weight (kg)	4.11 (2.22–7.19)	4.15 (3.23–6.08)	4.00 (2.22–7.19)	4.59 (3.23–7.65)
Creatinine concentration (mg/dL)	1.6 (1.0–2.0)	2.3 (2.0–4.0)	1.5 (0.9–2.0)	2.8 (2.0–4.0)
(μmol/L)	141 (88–177)	203 (177–354)	135 (81–177)	248 (177–354)

Results are presented as median (range).

Table 3. Extracellular fluid volume (ECFV) measurements in azotemic and nonazotemic cats. $ECFV_{Bromide}$ is extracellular fluid volume determined using bromide dilution. $ECFV_{Iohexol}$ is extracellular fluid volume determined from corrected slope-intercept GFR/ECFV using the filtration marker iohexol. BSA is body surface area determined using a formula based on body weight. Nonazotemic and azotemic cats were compared using the *t*-test.

	Nonazotemic Cats	n	Azotemic Cats	n	All Cats	n	*P*-Value
$ECFV_{Bromide}$ (L)	0.85 ± 0.20	55	0.82 ± 0.18	11	0.85 ± 0.19	66	.635
$ECFV_{Bromide}/BSA$ (L/m^2)	3.25 ± 0.45	55	3.12 ± 0.53	11	3.24 ± 0.46	66	.461
$ECFV_{Iohexol}$ (L)	0.85 ± 0.30	73	0.82 ± 0.40	16	0.84 ± 0.32	89	.774
$ECFV_{Iohexol}/BSA$ (L/m^2)	3.29 ± 0.90	73	2.95 ± 1.01	16	3.23 ± 0.92	89	.516

Mean ± SD presented.

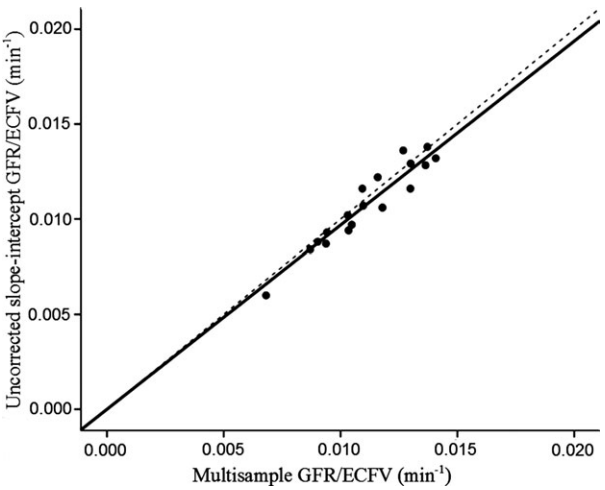

Fig 1. Relationship between multisample GFR/ECFV and slope-intercept GFR/ECFV. The relationship was linear. Bold line is the regression line for multisample GFR/ECFV and slope-intercept GFR/ECFV ($R^2 = 0.90$, $P < .001$) and dashed line is the line of equality.

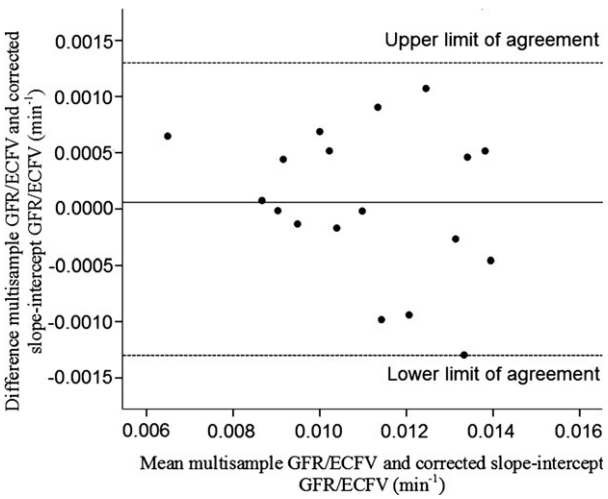

Fig 2. Bland-Altman agreement plot showing agreement between multisample GFR/ECFV and corrected slope-intercept GFR/ECFV in 18 nonazotemic cats. Bold line represents the bias (mean difference between two measurements) and dashed lines represent the upper and lower limits of agreement (mean difference between two measurements ± 2SD). Based on the negligible bias and narrow limits of agreement, agreement was considered excellent. Corrected slope-intercept GFR/ECFV was determined using the following equation: GFR/ECFV = 1.027 × β.

tion formula is presented in Table 3. $ECFV_{Iohexol}$ was not significantly different between azotemic and nonazotemic cats whether standardized to body size ($P = .177$) or unstandardized ($P = .774$). $ECFV_{Iohexol}$ determined from slope-intercept GFR/ECFV was weakly correlated with $ECFV_{Bromide}$ ($r = 0.385$, $P = .001$). Maximum difference between the two methods was 0.57 L which was >65% of mean ECFV for the group of cats. There were also several outliers particularly at larger ECFV. Agreement between the two methods was considered to be poor. This was based on the wide limits of agreement (−0.576 to 0.544 L; see Fig 3).

Standardization of ECFV Measurements

Appropriate methods of standardization were explored for $ECFV_{Bromide}$ and $ECFV_{Iohexol}$. A significant linear relationship was seen between BW and unscaled $ECFV_{Bromide}$ ($R^2 = 0.55$, $P < .001$) and $ECFV_{Iohexol}$ ($R^2 = 0.30$, $P < .001$). These relationships remained significant when both were scaled to BW (kg). A significant linear relationship was seen between BSA and $ECFV_{Bromide}$ ($R^2 = 0.60$, $P < .001$) and $ECFV_{Iohexol}$ ($R^2 = 0.29$, $P < .001$). However, no significant relationship was seen between either volume when standardized to BSA. Mean ± SD $ECFV_{Bromide}$ and $ECFV_{Iohexol}$ standardized to BSA are presented in Table 3. Relationships between $ECFV_{Bromide}$/BSA and $ECFV_{Iohexol}$/BSA and BSA are presented in Fig 4A,B.

Discussion

This study developed a cat specific correction factor for determining GFR/ECFV from slope-intercept iohexol clearance, thereby allowing determination of ECFV. ECFV determined using this method did not show good agreement with that determined using bromide dilution, although, this was predicted because of likely differences in the pharmacokinetics of the markers. In cats with mild azotemic CKD, there was no significant difference in ECFV compared to healthy nonazotemic cats. This suggests that fluid volume loss, as might be expected based on clinical findings in cats with late stage CKD or during acute on chronic episodes, or fluid volume overload, as can be seen in human patients with CKD, is not a prominent feature in cats with early stage CKD.

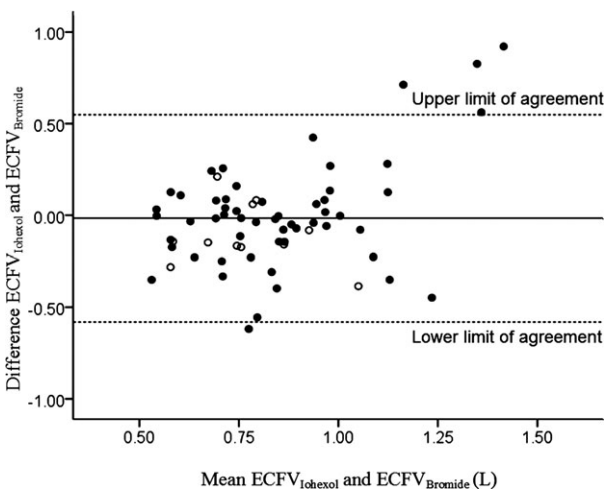

Fig 3. Bland-Altman agreement plot showing agreement between $ECFV_{Iohexol}$ and $ECFV_{Bromide}$ in 66 cats (55 nonazotemic and 11 azotemic). Bold line represents the bias (mean difference between two measurements of ECFV) and dashed lines represent the upper and lower limits of agreement (mean difference between two measurements of ECFV ± 2SD). Based on the negligible bias but wide limits of agreement, agreement was considered poor. Filled circles represent nonazotemic cats and unfilled circles represent azotemic cats.

A commonly reported method for determining volume of distribution of filtration markers in clearance studies is to calculate dose divided by concentration at time zero (determined from the intercept of the terminal elimination exponential, B). This overestimates true volume of distribution as marker is already lost through clearance between time of administration and completion of mixing within the distribution volume.[12] Determination of ECFV from the clearance curve using the methods described in this study is more appropriate. Results of the Bland-Altman agreement analysis suggest the feline correction formula for GFR/ECFV determined from slope-intercept clearance, provides an accurate estimate of multisample GFR/ECFV. However, the formula was developed and tested in the same group of cats and a separate group of cats for testing would be optimal. $ECFV_{Iohexol}$ is of importance as it can be obtained simultaneously with GFR determined from slope-intercept clearance in cats without the need for administration of an additional marker such as bromide. Knowledge of both ECFV and GFR could be important when assessing patients in different disease states including kidney disease and also before the use of renally cleared drugs or drugs which distribute within ECFV.

Poor agreement between ECFV determined using bromide dilution and iohexol clearance is not surprising. In human patients, ECFV determined from multisample plasma clearance of iohexol is reported to underestimate ECFV compared to bromide dilution.[19] There are a number of reasons which can explain this. First, iohexol is excreted from the body via the kidneys more rapidly than bromide.[19] Second, intracellular penetration of erythrocytes, leukocytes and some cells in the skin and gastric mucosa by bromide ions is known to occur.[1,20] It is therefore conventional practice to correct by a factor of 10% for intracellular penetration of the tracer. However, intracellular penetration of erythrocytes may vary because of variations in PCV, leading to over or underestimation of ECFV. Third, although previous studies have reported 120 minutes to be an appropriate sampling time for measuring ECFV using bromide dilution and that bromide will have fully equilibrated by this time,[8,9] it is possible that some cats could have had reduced distribution volumes and hence bromide might not have fully equilibrated. This would result in <10% penetrating intracellular compartments and more remaining in extracellular fluid leading to an underestimation in ECFV. Fourth, bromide and iohexol can differ in their measurements of ECFV because of the physiological properties of various compartments of ECFV such as plasma, interstitial and transcellular compartments, which might result in different penetration by markers. Indeed, a study of human patients demonstrated the distribution volume of the markers bromide and ^{99}Tc-DTPA to be inversely related to their molecular size with bromide having the largest distribution volume.[2] There was no consistent bias identified when performing agreement analysis between the two methods in this study, suggesting no consistent under or

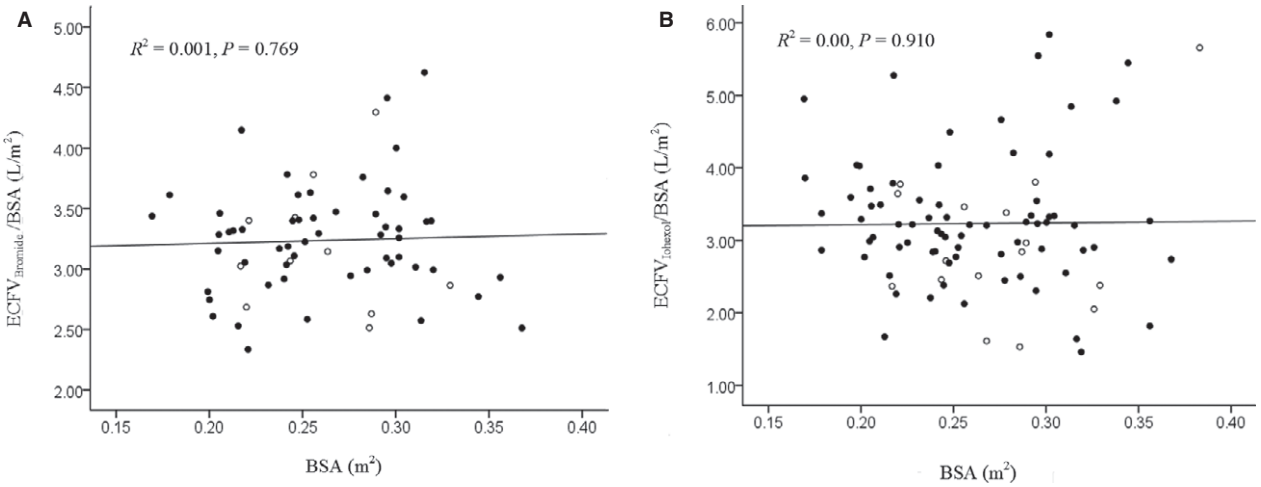

Fig 4. Relationship between **(A)** $ECFV_{Bromide}$/BSA and BSA in 66 cats (55 nonazotemic and 11 azotemic) and **(B)** $ECFV_{Iohexol}$/BSA and BSA in 89 cats (73 nonazotemic and 16 azotemic). Both relationships were nonsignificant indicating BSA is an appropriate method for normalization. Filled circles represent nonazotemic cats and unfilled circles represent azotemic cats. The relationships were evaluated by performing linear regression analysis and determining the coefficient of determination (R^2).

overestimation. Therefore, the poor agreement is likely related to the nonsystematic individual variation in pharmacokinetics of the tracer. It has been suggested in human patients that ECFV determined using iohexol clearance is a more reliable measurement than bromide dilution because of the intracellular penetration of bromide.[19]

Data regarding ECFV determined in this study suggest that estimates reported in textbooks[21] of ECFV representing approximately 20% of a healthy cat's BW, to be accurate in senior cats ($ECFV_{Bromide}$ and $ECFV_{Iohexol}$ 19% of BW). Furthermore, ECFV determined by bromide dilution (0.85 L) was similar to that reported in previous studies (0.93–1.01 L).[9,22] An increase in ECFV can occur in end stage renal disease in human patients and ECFV increases in the early stages of CKD by approximately 8%.[23] Human patients show a progressive increase in ECFV with declining GFR,[23,24] although there is no correlation between GFR and ECFV in human patients across a range of GFR.[25] In this study, there was no significant difference in ECFV between nonazotemic and azotemic cats.

Progressive kidney disease is characterized by an adaptive increase in sodium excretion per nephron.[26] As GFR declines and there is further nephron loss, sodium excretion per nephron might not be sufficient to meet the high sodium intake that is often characteristic of the diet eaten by human patients with CKD, particularly in the western world. Sodium balance and ECFV are closely associated and renal sodium excretion regulates ECFV. Therefore, patients with sodium retention would be expected to have expanded fluid volumes. In cats, sodium intake can be more controlled, particularly if fed a renal diet and this difference in diet could offer an explanation as to why ECFV was not increased in azotemic cats. In this study 81% of the azotemic cats were fed a renal diet. Data regarding formulation of the diet fed (wet or dry) were not reliably available for all cases and moisture content of the diet might be expected to influence ECFV. In contrast to human end stage renal patients, cats in the later stages of kidney disease are often considered to be clinically dehydrated.[27] No cats included in this study were in the advanced stages of CKD. Of the azotemic cats included, 6/11 could be classified as International Renal Interest Society (IRIS) stage 2 and 5/11 as IRIS stage 3. Further studies, including a larger number of cats and cats in more advanced stages of CKD are required to investigate the relationship between ECFV and renal disease.

Although it seems intuitive to assume that the relationship between multisample GFR and slope-intercept GFR would be the same as that of multisample GFR/ECFV and slope-intercept GFR/ECFV, as ECFV is a common denominator and should effectively cancel out, this is not the case. GFR is calculated as dose of marker administered divided by AUC. AUC for multisample GFR is determined as $A/\alpha + B/\beta$ and for slope-intercept GFR as B/β. The relationship between multisample GFR and slope-intercept GFR is nonlinear as the proportion of AUC which is missing (A/α) increases as GFR increases[10] requiring a polynomial correction factor. Multisample GFR/ECFV is determined from the reciprocal of MRT $((A/\alpha + B/\beta)/(A/\alpha^2 + B/\beta^2))$ and slope-intercept GFR/ECFV is determined from β. The relationship between slope-intercept GFR/ECFV (β) and multisample GFR/ECFV $((A/\alpha + B/\beta)/(A/\alpha^2 + B/\beta^2))$ in cats was found to be linear in this study and a second order polynomial did not improve the fit of the regression model. This relationship is in agreement with that of 50 dogs undergoing surgery for pyometra[28] but contradicts an earlier canine study in which the relationship was found to be nonlinear.[15] Slope-intercept GFR/ECFV has been shown to have a nonlinear relationship with multisample GFR/ECFV in human patients.[13,14] The reason for the difference in linearity of

the relationships is unclear, but could be because of the small number of cats included in this study. Slope-intercept GFR/ECFV corrected using the feline correction formula correlated with that corrected using the human formulae for ^{51}Cr-EDTA ($R^2 = 0.99$, $P < .001$) and iohexol ($R^2 = 0.99$, $P < .001$).[14] However, it was found that the canine formula[15] underestimated cat-corrected slope-intercept GFR/ECFV by approximately 0.32 min^{-1}. This underestimation is likely to be because of the inclusion of a constant (−0.326) in the canine correction formula. These relationships are of interest as it suggests the human correction formulae but not the dog formula could be an appropriate substitute for the feline correction formula for GFR/ECFV. This is in contrast to results using feline, canine and human correction formulae for GFR in cats.[10] It is important to note that a limitation of the feline correction formula is that it was derived and tested in the same population. This population consisted of only healthy nonazotemic cats and ideally would have contained cats with a greater range of GFR values.

In this study, BSA (m^2) but not BW (kg) was an appropriate method for standardizing fluid volume in cats. In human patients, ECFV correlated better with BSA than BW.[14] A limitation of standardization to BSA in cats is that the formula used to calculate BSA is based on BW and assumptions regarding shape and conformation are made. Moreover, the formula provides questionable accuracy.[16] Given the current lack of an alternative method, it is recommended that BSA is used for standardizing ECFV in cats.

In conclusion, this study developed a cat specific correction formula for determining GFR/ECFV using slope-intercept iohexol clearance which showed good agreement with multisample GFR/ECFV. This method provides both simultaneous assessment of renal function and also an estimate of ECFV. Agreement between ECFV determined from slope-intercept GFR/ECFV and ECFV determined using bromide dilution was poor. This appears to relate to individual differences between each cat which could reflect different penetration of the fluid compartment by the markers, variable intracellular penetration or nonsystematic individual variation in the pharmacokinetics of the tracers. It is recommended that if standardization of ECFV is required in cats, this should be to BSA. This study did not identify any significant difference in ECFV between azotemic and nonazotemic cats, suggesting loss of fluid volume or fluid volume overload is not an important clinical feature in mild CKD in cats.

Footnotes

[a] Mettler AM 100; Mettler-Toledo Ltd, Leicester, UK
[b] Health and Safety Laboratory, Buxton, UK
[c] Epsom and St Helier University Hospital NHS trust, Epsom, UK

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Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

References

1. Schoeller D. Hydrometry. In: Roche AFHS, Lohman TG, eds. Human Body Composition. Champaign, IL: Human Kinetics; 1996:25–44.

2. Cousins C, Skehan SJ, Rolph SM, et al. Comparative microvascular exchange kinetics of [(77)Br]bromide and (99 m) Tc-DTPA in humans. Eur J Nucl Med Mol Imaging 2002;29:655–662.

3. Watson AD, Lefebvre HP, Concordet D, et al. Plasma exogenous creatinine clearance test in dogs: Comparison with other methods and proposed limited sampling strategy. J Vet Intern Med 2002;16:22–33.

4. Sapirstein LA, Vidt DG, Mandel MJ, et al. Volumes of distribution and clearances of intravenously injected creatinine in the dog. Am J Physiol 1955;181:330–336.

5. Olsson B, Aulie A, Sveen K, et al. Human pharmacokinetics of iohexol. A new nonionic contrast medium. Invest Radiol 1983;18:177–182.

6. Mutzel W, Speck U. Pharmacokinetics and biotransformation of iohexol in the rat and the dog. Acta Radiol Suppl 1980;362:87–92.

7. Back SE, Krutzen E, Nilsson-Ehle P. Contrast media as markers for glomerular filtration: A pharmacokinetic comparison of four agents. Scand J Clin Lab Inv 1988;48:247–253.

8. Elliott DA. Evaluation of Multifrequency Bioelectrical Impedence Analysis for the Assessment of Body Composition in Cats and Dogs. University of California Davis; 2001. Thesis.

9. Elliott DA, Backus RC, Van Loan MD, et al. Extracellular water and total body water estimated by multifrequency bioelectrical impedance analysis in healthy cats: A cross-validation study. J Nutr 2002;132:1760S–1762S.

10. Finch NC, Syme HM, Elliott J, et al. Glomerular filtration rate estimation by use of a correction formula for slope-intercept plasma iohexol clearance in cats. Am J Vet Res 2011;72: 1652–1659.

11. Peters AM. Expressing glomerular filtration rate in terms of extracellular fluid volume. Nephrol Dial Transplant 1992;7:205–210.

12. Peters AM. The kinetic basis of glomerular filtration rate measurement and new concepts of indexation to body size. Eur J Nucl Med Mol Imaging 2004;31:137–149.

13. Bird NJ, Michell AR, Peters AM. Accurate measurement of extracellular fluid volume from the slope/intercept technique after bolus injection of a filtration marker. Physiol Meas 2009;30:1371–1379.

14. Bird NJ, Peters C, Michell AR, et al. Suitability of a simplified technique based on iohexol for decentralized measurement of glomerular filtration rate. Scand J Urol Nephrol 2008;42:472–480.

15. Gleadhill A, Peters AM, Michell AR. A simple method for measuring glomerular filtration rate in dogs. Res Vet Sci 1995;59:118–123.

16. Price GS, Frazier DL. Use of body surface area (BSA)-based dosages to calculate chemotherapeutic drug dose in dogs: I. Potential problems with current BSA formulae. J Vet Intern Med 1998;12:267–271.

17. Vaughan JA, Adams T. Surface area of the cat. J Appl Physiol 1967;22:956–958.

18. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–310.

19. Zdolsek JH, Lisander B, Hahn RG. Measuring the size of the extracellular fluid space using bromide, iohexol, and sodium dilution. Anesth Analg 2005;101:1770–1777.

20. Pierson RN Jr, Price DC, Wang J, et al. Extracellular water measurements: Organ tracer kinetics of bromide and sucrose in rats and man. Am J Physiol 1978;235:F254–F264.

21. DiBartola SP. Applied physiology of body fluids in dogs and cats. In: DiBartola SP, ed. Fluid, Electrolyte and Acid-Base Disorders in Small Animal Practice, 3rd ed. St Louis, MO: Elsevier; 2006:3–25.

22. Elliott DA, Backus RC, Van Loan MD, et al. Evaluation of multifrequency bioelectrical impedance analysis for the assessment of extracellular and total body water in healthy cats. J Nutr 2002;132:1757S–1759S.

23. Essig M, Escoubet B, de Zuttere D, et al. Cardiovascular remodelling and extracellular fluid excess in early stages of chronic kidney disease. Nephrol Dial Transplant 2008;23:239–248.

24. Vasavada N, Agarwal R. Role of excess volume in the pathophysiology of hypertension in chronic kidney disease. Kidney Int 2003;64:1772–1779.

25. Peters AM, Glass DM, Bird NJ. Extracellular fluid volume and glomerular filtration rate: Their relation and variabilities in patients with renal disease and healthy individuals. Nucl Med Commun 2011;32:649–653.

26. Shemin D, Dworkin LD. Sodium balance in renal failure. Curr Opin Nephrol Hypertens 1997;6:128–132.

27. Elliott J, Barber PJ. Feline chronic renal failure: Clinical findings in 80 cases diagnosed between 1992 and 1995. J Small Anim Pract 1998;39:78–85.

28. Heiene R, Moe L. The relationship between some plasma clearance methods for estimation of glomerular filtration rate in dogs with pyometra. J Vet Intern Med 1999;13:587–596.