To estimate GFR in cats, plasma clearance of the nonionic monomeric x-ray contrast medium iohexol is most commonly measured1 because measurement of the plasma clearance of the standard tracer inulin requires specialized technical procedures and inulin has low solubility that necessitates the collection of a large volume of blood. Use of a reduced iohexol dose (from 90 to 45 mg of I/kg) has been recommended for azotemic cats2 because there is a concern that iohexol can cause further deterioration of impaired renal function in humans,3 presumably because of its high osmolarity.

The isotonic nonionic dimeric x-ray contrast medium ioxixanol has potential for use as a new tracer to estimate GFR in cats. Iodixanol is rapidly excreted into urine without metabolic degradation and no or very little protein binding; it has a very short half-life,4,5 as does iohexol. However, results of randomized, double-blinded, prospective, multicenter studies involving high-risk human patients with chronic renal diseases have indicated that iodixanol is less nephrotoxic than nonionic contrast media, including iohexol. Chemically, iodixanol contains twice the amount of iodine in 1 molecule, compared with iohexol,4,5 and is assumed to have the same pharmacodynamic action at half the exposure dose to the body as iohexol.

The concentration of the tracer EDTA in a single plasma sample obtained a few hours after injection was reported to correlate with renal clearance in humans.7 In other words, the assumption made is that the extra-renal clearance of the tracer is negligible or at least constant and independent of renal clearance and therefore that plasma clearance of the tracer is a good estimate of its renal clearance.9 On the basis of a report by Jacobsson,8 we validated a single-sample method using iodixanol for estimation of GFR and evaluated the V and optimum time for collection of blood samples in rats in a previous study.9

The purpose of the study reported here was to compare the use of a single-sample method involving IV administration of iodixanol with a multisample method involving inulin for the estimation of glomerular filtration rate (GFR) in cats.

Objective—To compare the use of a single-sample method involving IV administration of iodixanol with a multisample method involving inulin for the estimation of glomerular filtration rate (GFR) in cats.

Animals—24 cats, including 15 healthy cats and 9 cats with naturally occurring renal diseases.

Procedures—Each cat was coadministered iodixanol (a nonionic contrast medium; dose providing 40 mg of I/kg) and inulin (50 mg/kg), IV, and blood samples were collected 60, 90, and 120 minutes later. Serum iodixanol and inulin concentrations were determined by means of high-performance liquid chromatography and colorimetry, respectively. Serum urea nitrogen and creatinine concentrations were also measured.

Results—Analysis of the data from healthy cats and cats with naturally occurring renal disease revealed an excellent correlation between GFR values estimated by the multisample and single-sample methods with iodixanol. Likewise, GFR values estimated from the single-sample method with iodixanol were closely correlated with those calculated from the multisample method with inulin.

Conclusions and Clinical Relevance—For estimation of GFR in cats, use of a single-sample method with iodixanol, instead of a multisample procedure, may be an expedient tool in both clinical and research settings because of its benefits to patient well-being as a result of reduced stress associated with blood sample collection. (Am J Vet Res 2012;73:1344–1349)
ing IV administration of iodixanol with a multisample method with inulin for the estimation of GFR in cats. Essentially the same protocol was used as that used for rats, but a scaled-up version was used for cats. First, GFR values determined via the multisample method with iodixanol were compared with values determined via the single-sample method with iodixanol in healthy cats and cats with naturally occurring renal diseases to examine whether the Jacobsson formula can be applied to cats. Next, GFR values determined by use of the single-sample method with iodixanol were compared with values determined by use of the multisample method with inulin.

**Materials and Methods**

**Animals**—Fifteen healthy purpose-bred cats (2 sexually intact females and 13 sexually intact males) and 9 cats with naturally occurring renal diseases (1 sexually intact female, 4 spayed females, 2 sexually intact males, and 2 castrated males) admitted to the Veterinary Teaching Hospital at Iwate University (Morioka, Japan) were included. The healthy cats were owned by the university, and cats with naturally occurring renal diseases were used after obtaining the owners’ consents for their participation in the present study. All experimental procedures were performed in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science and approved by the Animal Experimental Ethics Committee of Iwate University.

The mean age of healthy cats and cats with naturally occurring renal diseases was 1.3 years (1.0 years for females and 1.4 years for males) and 7.8 years (7.0 years for females and 8.8 years for males), respectively. The mean body weight of healthy cats and cats with naturally occurring renal diseases was 4.25 kg (3.18 kg for females and 4.91 kg for males) and 3.70 kg (3.88 kg for females and 3.48 kg for males), respectively. Renal disease was confirmed in the nonhealthy cats on the basis of clinical observations and clinicopathologic findings including results of hematologic evaluation, serum biochemical analysis, or urinalysis. In nonhealthy cats, mean SUN concentration was >11 mmol/L (reference range, 12 to 67 mmol/L) and serum creatinine concentration was 0.08 to 0.10 mmol/L, with no detectable proteinuria.

**Dosing protocol and blood sample collection**—To assess the appropriate dose for GFR estimation in cats, iodixanol was administered IV at a dose that provided 20, 40, 80, or 160 mg of I/kg in a cephalic vein of 4 healthy cats in a 4 × 4 crossover experiment at intervals of 10 days. A blood sample (0.4 mL) was collected with a 25-gauge needle from the contralateral cephalic vein before and at 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 minutes after injection of iodixanol.

Inulin (50 mg/kg) was given IV to another 4 healthy cats, and a blood sample (1.0 mL) was collected 60, 90, and 120 minutes later, in the manner described for the cats receiving iodixanol. The dosing regimen for inulin was selected on the basis of a previous report. In a preliminary experiment, when inulin was administered IV at 30 mg/kg to healthy cats (n = 2), serum inulin concentration 120 minutes later was less than the limit of quantification.

**Estimation of GFR**—To evaluate the correlation between GFR values obtained by the single-sample method with iodixanol and the multisample method with inulin, iodixanol (dose providing 40 mg of I/kg) and inulin (50 mg/kg) were coadministered IV to 15 healthy cats (4 cats had previously been used to determine the appropriate dose of iodixanol, and 4 additional cats had been used for the multisample study with coadministration of iodixanol and inulin) and 9 cats with naturally occurring renal diseases (total, 24 cats), and a blood sample (1.2 mL) was collected before dosing and 60, 90, and 120 minutes later. No difference was noted between GFR values estimated with iodixanol alone and with iodixanol and inulin in combination in the same healthy cats. This finding was not confirmed in cats with naturally occurring renal diseases. For repeated GFR measurements, the estimation was performed at an interval of at least 5 days. To assess potential adverse effects during or after the administration of iodixanol alone or in combination with inulin, cats underwent a physical examination and serum biochemical analyses after each dose administration.

**Analysis**—Serum iodixanol concentrations were measured by reverse-phase high-performance liquid chromatography according to a reported procedure, with minor modifications. Briefly, serum samples (100 µL) were deproteinized by adding 20% trichloroacetic acid at a ratio of 1:1 and stored at 4°C for 30 minutes to complete precipitation before removal of the proteins via centrifugation (14,000 × g at 4°C) for 10 minutes. The supernatant was centrifuged again under the same conditions. The high-performance liquid chromatography system was composed of separation equipment, a UV detector, and analytic software with a 250 × 4.6-mm C18 reverse-phase column. The stepwise mobile phase profile consisted of distilled water followed by 80% acetonitrile in distilled water, and the flow rate was maintained at 1 mL/min. The detection wavelength was 244 nm, which is the approximate maximum absorbance for iodixanol. The quantification limit of serum iodixanol concentration was 0.25 µg of I/mL. Validation studies revealed no significant difference between serum and plasma iodixanol concentrations.

Serum inulin concentrations were colorimetrically determined with a commercially available kit. The quantification limit of serum inulin concentration was 20 µg/mL. It was confirmed beforehand that there was no drug interference with either iodixanol or inulin concentration in serum in the in vitro additive studies. Serum urea nitrogen and creatinine concentrations were measured with an autoanalyzer on the same days that GFR was estimated.

**Calculation of GFR**—In the multisample method with iodixanol or inulin, clearance calculations were made on the basis of the 1-compartment or 2-compartment...
ment models. In brief, the AUC for iodixanol or inulin concentration was calculated via the linear trapezoidal rule with extrapolation by use of 3 to 11 sample points in serum, and the clearance value was calculated from the following formula:\textsuperscript{12}

\[ Cl = \frac{Q_{total}}{AUC} \]

where Cl is clearance and \( Q_{total} \) is the dose of iodixanol or inulin injected.

To determine iodixanol clearance for the single-sample method, the estimated \( V \) (\( V_1 \)) of iodixanol in each cat was back-calculated by substituting Cl values and serum iodixanol concentrations (\( C_{t1} \)) obtained via the multisample method at 60, 90, or 120 minutes into the following formula:\textsuperscript{8}

\[ Cl = \frac{1}{(t_1/V_1 + 0.0016) \times \ln (Q_{total}/(V_1 \times C_{t1}))} \]

where \( t_1 \) represents each sample collection time.

Because the \( V_1 \) value is dependent on elimination kinetics of each tracer and animal species, it is necessary to obtain that value in the respective study animals.\textsuperscript{9} The formula can be transformed to the following equation by the classic Newton method: \textsuperscript{13,14} and the variable \( b \) value can also be solved by the same method:

\[ V_1 = \frac{t_1 \times Cl}{b} \]

The \( V_1 \) value obtained was then reconfirmed with spreadsheet software.\textsuperscript{1}

To examine the estimated \( V \) in each cat, an equation between the \( V_1 \) values and serum iodixanol concentration at 60, 90, and 120 minutes was determined from a scatterplot. Finally, the GFR value estimated by the single-sample method with iodixanol was measured by substituting the estimated \( V \) (\( V_1 \)) value calculated, the iodixanol dose (ie, 40 mg of I/kg), and serum iodixanol concentration at 90 minutes for each cat into the following Jacobsson formula:\textsuperscript{8}

\[ GFR = \frac{1}{(t/V + 0.0016) \times \ln (Q_{total}/(V \times C_{90}))} \]

where \( t \) is time and \( C_{90} \) is the serum iodixanol concentration at 90 minutes. The Cl term was regarded as the GFR for the present study.

The GFR was represented (as mL/min/m\(^2\)) on the basis of the following equation for body surface area\textsuperscript{15}
because of a large variation in the body weight (kg) of cats used:

\[ \text{Body surface area} = 0.10 \times \text{body weight}^{0.67} \]

Statistical analysis—Quantitative data are expressed as the mean ± SEM of each group. Statistical comparisons between 2 groups were performed with ANOVA via the \( F \) test followed by Student \( t \) test. Differences among >2 groups were compared via the Dunnett test. Values of \( P < 0.05 \) were considered significant. Comparison of GFR values between the 2 methods was performed according to standard recommendations for comparing analytic techniques on the basis of Deming regression analysis and Bland-Altman bias presentation\textsuperscript{6,11} with statistical software.\textsuperscript{3}

Results

Appropriate dose of iodixanol and blood sample collection times for estimation of GFR—In 4 healthy cats administered iodixanol IV at a dose of 20, 40, 80, or 160 mg of I/kg, mean serum iodixanol concentrations decreased linearly from 60 to 240 minutes after iodixanol dosing at 40, 80, or 160 mg of I/kg and by 150 minutes after iodixanol dosing at 20 mg of I/kg (data not shown). Considering the detection sensitivity and minimum exposure of the whole body to iodixanol, an iodixanol dose providing 40 mg of I/kg iodixanol was chosen for use in the remainder of the study (Figure 1). With the iodixanol dose at 40 mg of I/kg, there was a significant (\( P < 0.01 \)) difference between GFR values estimated from the 1-compartment (56.1 ±

![Figure 1](link)

![Figure 2](link)
2.7 mL/min/m²) versus the 2-compartment (48.7 ± 0.8 mL/min/m²) model, whereas no difference (P > 0.001) was detected between GFR values estimated from 7 versus 3 blood-sample collection times (37.0 ± 1.3 mL/min/m²) in the 1-compartment model. For subsequent investigations in the present study, a combination of iodixanol (dose providing 40 mg of l/kg) with blood sample collection times at 60, 90, and 120 minutes after dosing was selected.

Estimation of the GFR by the single-sample method with iodixanol—The equation for calculating the estimated V value was determined from a scatter diagram (n = 24 cats; number of samples, 62 [sum of the GFR values obtained for the same cats on different days]; Figure 2) as follows:

\[ V = 647.6e^{-0.023C} \]

where C is serum iodixanol concentration at 90 minutes after dosing; analysis between estimated the V value and serum iodixanol concentration revealed a close correlation (r = 0.95; P = 0.001). Serum iodixanol concentration at 90 minutes after dosing was chosen because of the high correlation between V1 values and serum inulin concentrations, compared with that (r = 0.90 to 0.92) determined at 60 or 120 minutes after dosing. By use of iodixanol, a comparison of GFR values obtained by the multisample method with those determined by the single-sample method yielded a strong correlation (P < 0.001; Figure 3).

Correlation between GFR determined by the multisample method with inulin and that determined by the single-sample method with iodixanol—A close correlation (P < 0.001) was identified between the GFR values estimated by the 2 methods for 20 cats (35 samples; Figure 4). In the present study, no adverse clinical signs were observed in any of the cats during or after the administration of iodixanol alone or in combination with inulin, as determined on the basis of results of physical examinations and serum biochemical analyses.

Relationship between GFR determined by the single-sample method with iodixanol versus SUN or serum creatinine concentration—The relationship between GFR determined by the single-sample method and SUN or serum creatinine concentration was assessed for 24 cats (62 samples). When the GFR decreased to >70% of the basal reference level (mean GFR, 57.5 mL/min/m²), serum creatinine concentrations likely began to increase (Figure 5). In contrast, SUN concentration fluctuated to some extent.
Discussion

The tolerability of iodixanol in mice, rats, rabbits, and monkeys has been reported to be extremely high in various studies.\(^8\) After serial iodixanol administrations to azotemic cats under the protocols of the present study, no serum iodixanol residue was detected prior to dosing.

In the multisample method with iodixanol, the appropriate combination was considered to be an iodixanol dose that provided 40 mg of I/kg with blood sample collection after 60, 90, and 120 minutes. This regimen resulted in a linear semilogarithmic plot of serum iodixanol concentrations versus time, indicating the suitability of use of the 1-compartment model for clearance estimation. The IV injectable error can be verified by observing the shape of the elimination curve.\(^9\)

The 1-compartment model was thought to underestimate AUC, compared with the 2-compartment and noncompartment models, and the AUC for serum iodixanol concentration obtained from the 1-compartment model in the present study was also approximately 13% to 18% lower than that from the 2-compartment model, indicative of higher GFR in the 1-compartment model. However, no significant difference was detected between the GFR values estimated from 7 versus 3 blood sample collection times in the 1-compartment model. On the basis of these results, the approach involving 3 blood sample collection times was chosen because of a minimum SE for difference in GFR values. Therefore, we focused on the formula described by Jacobsson,\(^8\) including the iodixanol dose, V, and serum concentration of iodixanol concentration and sample collection time as variable factors. The formula derived for the GFR estimation with 1 sample was reported to require that the V value be known,\(^8\) and the accuracy in the V value determines the accuracy in the method. Moreover, the formula combined with the V value and optimum time is recognized to yield an accurate GFR estimate.\(^8\) If the V value of the tracer is known, a plasma disappearance curve can be closely approximated from a single, timed measurement of plasma tracer concentration.\(^9\) In the present study, the estimated V value of individual cats was determined by substituting the GFR value obtained from the multisample method and serum iodixanol concentration at 90 minutes into the formula described by Jacobsson.\(^8\) The V value could be completely solved by the Newton method.\(^13,14\) A close correlation was observed between GFR values obtained from the multisample and single-sample methods with iodixanol, suggesting that the single-sample method can be used to estimate GFR in cats as an alternative to the multisample method. These data indicated that the formula described by Jacobsson can be applied to healthy cats and cats with naturally occurring renal diseases.

In the single-sample method with iodixanol, the mean GFR value for cats was calculated on the basis of body weight as 3.36 mL/min/kg (2.80 to 3.92 mL/min/kg). These basal values were similar to previously reported GFR data,\(^1,10,22\) although tracers and experimental conditions were markedly different.

On the basis of the data collected from healthy cats and cats with naturally occurring renal diseases, serum creatinine concentrations likely began to increase from the point at which the GFR values decreased to > 70% of the basal value, whereas SUN concentration fluctuated greatly. These phenomena may be explained by the notion that renal handling of urea nitrogen is different from that of creatinine (ie, creatinine is filtered and elicits only slight secretion, whereas urea nitrogen is reabsorbed in the proximal tubule and flow rate in that nephron segment greatly affected its reabsorption). Thus, SUN concentrations were considered to vary more than serum creatinine concentrations in association with changes in GFR.

For estimation of GFR in cats, the advantages of a bolus IV injection of iodixanol and collection of a single blood sample are that fewer analytic procedures are required and the exact timing of blood sample collection is unnecessary. However, further studies are necessary to collect cumulative background data, including the effects of sex, age, dietary protein intake, hydration status, sodium balance, and circadian rhythm for application of this procedure to feline veterinary practice.

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