**Comparison of serum iohexol clearance and plasma creatinine clearance in clinically normal horses**

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**Objective**—To determine whether a limited sampling time method based on serum iohexol clearance (Cl<sub>iohexol</sub>) would yield estimates of glomerular filtration rate (GFR) in clinically normal adult horses similar to those for plasma creatinine clearance (Cl<sub>creatinine</sub>).

**Animals**—10 clinically normal adult horses.

**Procedures**—A bolus of iohexol (150 mg/kg) was administered IV, and serum samples were obtained 5, 20, 40, 60, 120, 240, and 360 minutes after injection. Urinary clearance of exogenous creatinine was measured during three 20-minute periods. The GFR determined by use of serum Cl<sub>iohexol</sub> and plasma Cl<sub>creatinine</sub> was compared with limits of agreement plots.

**Results**—Values obtained for plasma Cl<sub>creatinine</sub> ranged from 1.68 to 2.69 mL/min/kg (mean, 2.11 mL/min/kg). Mean serum Cl<sub>iohexol</sub> was 2.38 mL/min/kg (range, 1.95 to 3.33 mL/min/kg). Limits of agreement plots indicated good agreement between the methods.

**Conclusions and Clinical Relevance**—Use of serum Cl<sub>iohexol</sub> yielded estimates of GFR in clinically normal adult horses similar to those for plasma Cl<sub>creatinine</sub>. This study was the first step in the evaluation of the use of serum Cl<sub>iohexol</sub> for estimating GFR in adult horses. (Am J Vet Res 2009;70:1545–1550)
a constant rate infusion to achieve steady-state conditions. Steady-state conditions may be mimicked through SC injection of creatinine and its subsequent absorption. Exogenous Cl\textsubscript{creatinine} has been safely evaluated via SC injection in dogs\textsuperscript{2} and healthy horse foals. Although theoretically accurate, methods for determining Cl\textsubscript{creatinine} in horses are time-consuming procedures and difficult to perform. Urinary clearance evaluations necessitate collection of the total amount of urine produced by an animal during a specified time period. Total urine collection should ideally be performed by catheterization of the ureters to prevent omission of part of the urine volume in the urinary bladder. This technique is challenging and not practical for clinical purposes. Catheterization of the urinary bladder of adult horses is a simple procedure, but long-term maintenance of catheters is problematic, and collection of total urine volume is not ensured. Catheters may become dislodged from the bladder and are a risk factor for induction of urinary tract infection.

Iohexol is a nonionic compound of low osmolality. It is used most commonly in humans and other animals as a radiographic contrast agent for urography, contrast-enhanced computed tomography, and angiography. Intravenous injection of iohexol is not associated with adverse effects, even in humans and other animals with renal insufficiency. Once injected, iohexol is not metabolized by the body, bound to plasma proteins, or secreted or absorbed by the renal tubules; it is freely filtered at the glomerulus, which makes it a useful marker for GFR evaluations.\textsuperscript{3}

The Cl\textsubscript{creatinine} has been used to estimate GFR in humans,\textsuperscript{4,5,7,8} dogs,\textsuperscript{9,10,12} cats,\textsuperscript{11,13–18} pigs,\textsuperscript{19} sheep,\textsuperscript{20} and foals.\textsuperscript{21} It is a safe and easy method that yields reproducible estimates of GFR, when compared with use of the inulin clearance and Cl\textsubscript{creatinine} techniques. In clinically normal foals, GFR determined by use of Cl\textsubscript{creatinine} agrees with GFR determined by use of exogenous Cl\textsubscript{creatinine}.\textsuperscript{7} Results of that study\textsuperscript{7} indicate that a single IV injection of iohexol followed by acquisition of 2 serum samples at 4 and 6 hours after injection can be used to estimate GFR in healthy foals. The objective of the study reported here was to determine whether a limited sampling time method based on serum Cl\textsubscript{creatinine} would yield estimates of GFR in horses similar to those determined by use of plasma Cl\textsubscript{creatinine}.

### Materials and Methods

**Animals**—Ten adult horses (6 mares and 4 geldings) were used in the study. Horses were 6 to 21 years old and weighed between 436 and 682 kg. Breeds included Thoroughbred (n = 4), American Quarter Horse (3), warmblood-crossbred horse (1), Arabian (1), and Morgan (1). All horses were healthy as determined on the basis of results of physical examination, a CBC, serum biochemical analysis, and urinalysis. Horses were housed separately in stalls with access to a small turnout area. Horses were provided grass hay and water ad libitum during the study. Physical variables were monitored in each horse every 6 hours for at least 24 hours after completion of the procedures. All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee.

**Preparation of animals**—A sterile 14-gauge, 5.5-inch catheter\textsuperscript{2} was aseptically inserted in the left and right jugular veins of each horse. Geldings were sedated by IV administration of 0.5 mg of xylazine hydrochloride/kg to facilitate aseptic placement of a 100-cm, 28-F Foley catheter in the urinary bladder. Mares were not sedated prior to placement of a 30-cm, 24-F Foley catheter in the urinary bladder. Thirty milliliters of sterile saline (0.9% NaCl) solution was instilled in the balloon of each Foley catheter to ensure maintenance of the catheters within the urinary bladder during the study period. Geldings were allowed at least 3 hours for elimination of xylazine prior to initiation of the experiments.

**Serum Cl\textsubscript{creatinine}—**Iohexol (150 mg/kg) was injected IV as a bolus via the catheter in the right jugular vein. Time 0 corresponded to the time of completion of injection of the iohexol bolus and simultaneous SC injection of 65% of the creatinine dose. After injection, the catheter in the right jugular vein was flushed with heparinized saline solution and removed. Blood samples were collected via the catheter in the left jugular vein 5, 20, 40, 60, 120, 240, and 360 minutes after iohexol injection. A standardized procedure was used for collection of blood samples. The catheter was flushed with 6 mL of heparinized saline solution, and 10 mL of blood was aspirated from the catheter. Approximately 10 mL of blood was aspirated from the catheter and immediately placed into a serum tube, and the catheter then was flushed with 6 mL of heparinized saline solution. The catheter was removed from the left jugular vein after collection of the sample at 360 minutes. Each serum tube was labeled with the time of blood collection and the horse’s name and allowed to clot at room temperature (22°C) for at least 2 hours. The tubes of blood were then centrifuged (1,000 \texttimes g) at 22°C for 5 minutes, and approximately 3 mL of serum was harvested from each tube. Serum samples were divided into 2 aliquots and placed in plastic vials, which were frozen at −70°C. Frozen samples were sent to an animal health diagnostic laboratory\textsuperscript{7} for analysis.

Iohexol concentration in serum samples was determined via high-performance liquid chromatography by use of a method described in 1 study\textsuperscript{22} as modified in another study.\textsuperscript{14} Equipment included a separations module\textsuperscript{24} with a dual-absorbance detector\textsuperscript{25} at 254 nm and a 125 \texttimes 4.6-mm, 5-μm octadecysilane column.\textsuperscript{8} The detection limit was 5 mg of iohexol iodine/mL, and the limit of quantification in serum was 15 mg of iohexol/L.

**Plasma Cl\textsubscript{creatinine}—**Aseptic techniques were used to prepare a creatinine solution\textsuperscript{25} by dissolving 1 g of creatinine/12 mL of lactated Ringer’s solution. Final concentration of the creatinine solution was 80 mg/mL. Creatinine solution (dose, 60 mg/kg) was prepared for each horse and stored in sterile glass containers for ≤18 hours prior to injection. Simultaneously with iohexol injection, 65% of a horse’s total creatinine dose was injected SC in the axillary, pectoral, and caudal cervical areas. Time 0 corresponded to completion of injection of iohexol and this first injection of creatinine. To minimize the number of injection sites needed, the
largest volume of creatinine solution possible was injected into an area until the horse displayed signs of discomfort. Twenty-five minutes later, the remaining 33% of the creatinine dose was injected SC in the axillary, pectoral, and caudal cervical areas. Immediately after the remaining 33% of the creatinine dose was injected, the bladder of the horse was emptied and washed with sterile saline solution to ensure removal of all urine. A clamp was then placed on the urinary catheter to retain all urine produced during the collection period. Blood collection was performed by use of the same technique described for the iohexol blood samples, except that 6 mL was collected and placed in heparinized blood tubes at each time point. Forty-five minutes after completing the creatinine injections, urine was collected from the bladder. The bladder was washed 3 times with 500 mL of saline solution/wash, and all fluid recovered was added to the collected urine. Total volume was recorded, and 2 mL of urine-wash mixture was placed in a sterile tube for measurement of the urine creatinine concentration. A blood sample (6 mL) was obtained from the jugular vein catheter for determination of plasma creatinine concentration. Then the urinary catheter was again clamped until the subsequent urine collection. Urine collection was repeated 65 and 85 minutes after creatinine injection, and blood samples were collected at those time points for determination of plasma creatinine concentrations. After collection of urine at 85 minutes, the urinary catheter was removed.

Plasma and urine creatinine concentrations were determined with an automated analyzer via a kinetic modification of the Jaffe method. The Clcreatine was calculated for each time interval by use of the following equation:

$$Cl_{creatinine} = \frac{\text{urine volume} \times \text{creatinine}_{\text{urine}}}{\text{creatinine}_{\text{plasma}} / \text{body weight}}$$

where creatinine_{urine} is the creatinine concentration in urine, and creatinine_{plasma} is the creatinine concentration in plasma. Comparisons with Cl_iohexol were made by use of the mean of the 3 time points for each horse.

Iohexol pharmacokinetic calculations—Monoexponential, biexponential, and triexponential equations were analyzed by use of nonlinear least squares regression analysis with equal weighting of the data via commercial software. The following triexponential equation described the data for each horse:

$$C_S = (C_1 \times e^{-\lambda_1 t}) + (C_2 \times e^{-\lambda_2 t}) + (C_3 \times e^{-\lambda_3 t})$$

where $C_S$ is the serum concentration at any time (t), $C_1$, $C_2$, and $C_3$ are concentration intercepts for the distribution phase, $C_i$ is the concentration intercept for the postdistribution phase, $\lambda_1$ and $\lambda_2$ are slopes of the distribution phase curve, and $\lambda_3$ is the slope of the postdistribution phase curve. The AUC was calculated from the intercepts and slopes of the triexponential equations for each horse by use of the equation $AUC = (C_1 / \lambda_1) + (C_2 / \lambda_2) + (C_3 / \lambda_3)$. Total serum clearance was calculated as dose/AUC.

Statistical analysis—Clearance values were expressed as milliliters per minute per kilogram and reported as the mean. Analyses of serum concentration-versus-time profiles were performed for each horse in the study. Analysis was performed by use of commercial software on a personal computer. The Cl_iohexol and Clcreatine were compared to assess agreement between the 2 methods. A paired t test was used to test for mean bias between methods, and proportional bias was evaluated by plotting the differences between mean values of both methods, as described by Bland and Altman. The SD of the difference was calculated, and limits of agreement were set and declared significant at $P \leq 0.05$. An ANOVA was performed to compare the AUC of the 3-compartment model with that of the 2-point estimates after calculating the terminal slopes extracted from the model at 3 and 4 hours, 4 and 6 hours, and 3 and 6 hours. The correction factor used to predict Cl_iohexol from the value for Cl_3-4hours was derived by use of errors in variables regression.

Results

Plasma Clcreatine—Baseline plasma creatinine concentration for all horses ranged from 0.9 to 1.3 mg/dL. Forty-five minutes after completing the creatinine injections, plasma creatinine concentrations ranged from 3.8 to 6.8 mg/dL. Values obtained for plasma Clcreatine ranged from 1.68 to 2.69 mL/min/kg (mean, 2.11 mL/min/kg).

Serum Cl_iohexol—After IV injection of iohexol, mean serum iohexol concentrations ranged from 961.18 mg/mL at 5 minutes to 17.77 mg/mL at 360 minutes. The

Figure 1—Mean ± SD semilogarithmic serum concentration of iohexol versus time in 10 horses after IV administration of a single dose (150 mg/kg). Time 0 corresponds to the time of completion of injection of the iohexol bolus and simultaneous SC injection of 65% of the creatinine dose. The solid line represents values calculated by use of 2-point estimates.

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Table 1—Pharmacokinetic values describing the disposition of iohexol in 10 horses after IV administration of a single dose (150 mg/kg).

<table>
<thead>
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<th>Variable</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
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<tbody>
<tr>
<td>Cl (µg/mL)</td>
<td>471.7</td>
<td>306.0</td>
<td>543.2</td>
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<td>C1 (µg/mL)</td>
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<td>136.5</td>
<td>754.4</td>
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<td>Cl (µL/min/kg)</td>
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<td>61.5</td>
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<td>λ1 (min⁻¹)</td>
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<td>0.025</td>
<td>0.236</td>
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<tr>
<td>λ2 (min⁻¹)</td>
<td>0.0192</td>
<td>0.015</td>
<td>0.084</td>
</tr>
<tr>
<td>λ3 (min⁻¹)</td>
<td>0.0062</td>
<td>0.0046</td>
<td>0.0115</td>
</tr>
<tr>
<td>AUC (µg/mL•h)</td>
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<td>45,071.1</td>
<td>77,039.9</td>
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<tr>
<td>Vc (L/kg)</td>
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<td>0.1064</td>
<td>0.1548</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>0.3675</td>
<td>0.2824</td>
<td>0.5197</td>
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</table>

The equation describing the 3-compartment model is as follows: 

\[ C_s(t) = C_s(0)e^{-\lambda_1t} + C_s(0)e^{-\lambda_2t} + C_s(0)e^{-\lambda_3t}, \]

where \( C_s(t) \) is the serum concentration at any time \( t \), \( C_s(0) \) is the serum concentration at time 0, and \( \lambda \) is the terminal slope calculated from each of the 2-point combinations. Use of an ANOVA to compare the AUCs for each of the curves generated from each of the 2-point estimates revealed no significant differences among the AUCs. The 2-point model for samples obtained at 3 and 4 hours was chosen because it was not significantly different from the other models, and the sample times were thought to be most clinically practical and convenient. Errors in variables regression for the AUCs of the 2-point model at 3 and 4 hours and the 3-compartment model were performed (Figure 2). The following equation was generated: 

\[ \text{AUC}_{\text{corrected}} = (1.107716 \times \text{AUC}_{\text{3-4hours}}) + 15.731, \]

where \( \text{AUC}_{\text{corrected}} \) is the corrected AUC, and \( \text{AUC}_{\text{3-4hours}} \) is the AUC determined by use of the 2-point estimates for samples obtained at 3 and 4 hours. Predicted clearance was calculated for each horse as dose/AUC_{corrected}.

**Comparison of Cl\textsubscript{iohexol} and Cl\textsubscript{creatinine}**—Values for Cl\textsubscript{creatinine} and Cl\textsubscript{iohexol} for the 3-compartment model, and Cl\textsubscript{iohexol} and Cl\textsubscript{creatinine} 3-4 hours were compared. For Cl\textsubscript{creatinine} versus Cl\textsubscript{iohexol}, Cl\textsubscript{creatinine} versus Cl\textsubscript{iohexol} 3-4 hours, and Cl\textsubscript{iohexol} versus Cl\textsubscript{creatinine}, the mean of the 2 methods was plotted against the difference between the 2 methods as described by Bland and Altman (Figure 3). A paired t test was performed between the means of each method. The Cl\textsubscript{creatinine} was significantly different from Cl\textsubscript{iohexol} and Cl\textsubscript{iohexol} 3-4 hours (\( P = 0.01 \) and \( P = 0.02 \), respectively). There was no significant difference between Cl\textsubscript{iohexol} and Cl\textsubscript{creatinine}.

**Discussion**

Evaluation and monitoring of renal function should be a standard of practice in the prevention, treatment, and monitoring of renal damage, regardless of whether it is primary or secondary to systemic disease, toxins, or drug administration. Commonly used methods of assessing renal function, such as determination of SUN or creatinine concentrations and urine specific gravity, are simple to perform and readily available to practitioners, but they are insensitive for determining early or mild renal dysfunction. Use of creatinine and SUN concentrations to estimate GFR is unsatisfactory and may lead to delays in diagnosis and treatment of renal disease. Values for fractional excretion of electrolytes are also easy to determine but may be substantially affected by nonrenal factors, which complicates the interpretation of results. Methods for determining clearance can be accurate and precise, but they are time-consuming, require specialized equipment and trained personnel, involve costly substances and assays, and leave much room for technical error. However, GFR is the best overall measurement of kidney function and the measurement most easily understood by clinicians.

Iohexol meets the requirements of a marker for measurement of GFR because it is freely filtered at the
glomerulus, is neither secreted nor reabsorbed by the kidneys, is not substantially bound to proteins or metabolized, and is not toxic.\(^6\) Comparison of CL\(_{\text{iophexol}}\) with CL\(_{\text{creatinine}}\) should determine the usefulness of the former as an assessment of renal function. Clearance values determined in this study for both CL\(_{\text{iophexol}}\) and CL\(_{\text{creatinine}}\) were within reference intervals for GFR in adult horses determined by a variety of methods.\(^1,2,7-30\) Serum CL\(_{\text{iophexol}}\) is a safe and reliable assessment of GFR in other species, including humans, and in clinically normal foals.\(^3,6-23\) The technique involves use of a commercially available, safe, and easy-to-use product and assay. Additionally, this technique avoids the time-consuming and error-prone necessity of collecting urine.

The significant difference between the means of the 2 methods was unexpected. On the basis of the more complicated nature and limitations of the method for determining CL\(_{\text{creatinine}}\), we suspect that serum CL\(_{\text{iophexol}}\) may be a more accurate measurement of GFR. To determine the validity of serum CL\(_{\text{iophexol}}\) as a measurement of GFR, it should be compared with a more accurate measurement of GFR, such as inulin clearance. However, because of the limited availability of inulin and its assay along with the complicated technique, the assessment of inulin clearance is not suitable for clinical practice. By default, CL\(_{\text{creatinine}}\) has been used as a determinant of GFR in horses. Because the objective of the study reported here was to determine a clinical technique to replace the use of CL\(_{\text{creatinine}}\), the results serve to compare these 2 methods but cannot be used to verify accurate determination of GFR in horses.

Most studies in which investigators have compared methods of assessment of GFR in humans or horses have used correlation analysis to determine the strength of the relationship between 2 methods. Bland and Altman\(^26\) have described a method to measure the agreement between 2 methods whereby the differences between methods are plotted against their mean. Limits of agreement are calculated as the 95% confidence interval of the mean difference. The limits should be interpreted with respect to the clinical range of the product or compound that is being measured. Results of the study reported here revealed narrow limits of agreement that were within the reference ranges for estimates of GFR in adult horses and, thus, good agreement between the methods.

A full 3-compartment pharmacokinetic analysis is not practical in clinical patients because it necessitates frequent timed collection of numerous blood samples and costly assays. To determine a more clinically useful method for estimation of GFR, limited sampling times were chosen and clearance was calculated on the basis of models for them. Results for all of the models created by the elimination curve formed by 2 terminal time points agreed significantly with results for the 3-compartment model for CL\(_{\text{iophexol}}\). Because results for the 3- and 4-hour sampling point agreed significantly with results for the 3-compartment model for CL\(_{\text{iophexol}}\), the 3-compartment model was used in future studies.

In humans and other animals, IV administration of iohexol appears to be safe and iohexol fulfills all of the requirements of a marker for GFR. The nonionic composition of iohexol and its low osmolality make it a stable and safe compound, even in patients with renal insufficiency.\(^31\) No adverse effects of iohexol administration were seen in the horses in our study.

Determining CL\(_{\text{iophexol}}\) is a technically simple procedure and has a number of advantages, compared with other clinical methods of measuring GFR in horses. First, it avoids the necessity of timed collection of urine samples. In 2 geldings used during this study, problems were encountered in the maintenance and patency of the urinary catheter and urine could not be collected. These horses were excluded from the study. Urine collection techniques necessitate collection of the total amount of urine produced over a specified period, usually 24 hours. The time and personnel required for such procedures make such techniques impractical for clinical use. The large size of the equine bladder and its ventral location in mares make it difficult, if not impossible, to ensure collection of total urine volume. Measurement of serum CL\(_{\text{iophexol}}\) avoids all of these difficulties.

Figure 3—Bland-Altman plot to compare CL\(_{\text{iophexol}}\) versus CL\(_{\text{creatinine}}\) in 10 horses. Clearance values were calculated as the mean of the 2 methods and plotted against the difference between the 2 methods. Positive values indicate that CL\(_{\text{iophexol}}\) exceeded CL\(_{\text{creatinine}}\). Each black circle represents results for 1 horse. The dashed line represents the mean difference, and the dotted lines represent the limits of agreement.
Other markers, including radiolabeled pharmaceuticals such as $^{99m}$Tc-DTPA, have been used to estimate GFR.27,30-33 Studies27,30-33 in horses have revealed good correlation between these methods and inulin clearance. However, these methods require specialized and careful handling of the compounds and animals, and they are expensive to perform, which limits their use in clinical practice to facilities with the necessary equipment.

Plasma $C_i_{\text{creatinine}}$ was chosen as a clinical standard of measurement of GFR for comparison with $C_i_{\text{iohexol}}$ for this study. Although $C_i_{\text{creatinine}}$ is an accurate and reliable method for use in estimating GFR in horses, the technique is fraught with potential error, as mentioned previously. To better assess the accuracy of $C_i_{\text{iohexol}}$ for use as a measure of GFR, the technique may be compared with more accurate, but more clinically impractical, methods such as inulin or $^{99m}$Tc-DTPA clearance. Further studies are also necessary to determine estimation of GFR by use of serum $C_i_{\text{iohexol}}$ in horses with evidence of renal dysfunction or failure.

References

11. Gaspari F, Perico N, Matalone M, et al. Precision of plasma clear-

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