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 ($Cl_{iohexol}$) would yield estimates of glomerular filtration rate (GFR) in clinically normal horses similar to those for plasma creatinine clearance ($Cl_{creatinine}$).

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 $Cl_{iohexol}$ and plasma $Cl_{creatinine}$ was compared with limits of agreement plots.

Results—Values obtained for plasma $Cl_{creatinine}$ ranged from 1.68 to 2.69 mL/min/kg (mean, 2.11 mL/min/kg). Mean serum $Cl_{iohexol}$ 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 $Cl_{iohexol}$ yielded estimates of GFR in clinically normal adult horses similar to those for plasma $Cl_{creatinine}$. This study was the first step in the evaluation of the use of serum $Cl_{iohexol}$ for estimating GFR in adult horses. (*Am J Vet Res* 2009;70:1545–1550)

Renal dysfunction in horses may develop secondary to changes in hemodynamics, intrinsic renal disease, or postrenal disease. Serum creatinine and SUN concentrations are the indices of renal function most commonly used as measurements of renal retention of nitrogenous wastes. However, because of the extensive reserve capacity of the kidneys, changes in SUN and creatinine concentrations are not evident until GFR has been reduced by approximately 75%. Accurate estimation of GFR allows determination of early, small changes in renal function.

Quantitative measures of renal function can be categorized as plasma disappearance curves or clearance evaluations involving timed urine collections. The 2 techniques involve measurement of an endogenous or exogenous substance and its disappearance from the plasma, appearance in the urine, or both. The traditional standard for measurement of GFR is inulin clearance. However, neither inulin nor its assay is readily available commercially, which makes this method impractical for clinical or research purposes.

Abbreviations

^{99m}Tc-DTPA	Technetium Tc 99m diethylenepentaacetic acid
AUC	Area under the concentration versus time curve
$Cl_{3\text{-}4hours}$	Clearance determined from 2-point estimates after calculating the terminal slopes extracted from the model at 3 and 4 hours
$Cl_{creatinine}$	Creatinine clearance
$Cl_{iohexol}$	Iohexol clearance
GFR	Glomerular filtration rate

Creatinine meets the requirements for a marker of glomerular filtration because it is neither secreted nor reabsorbed by the renal tubules in horses.[1] However, use of endogenous $Cl_{creatinine}$ frequently underestimates GFR in horses[1,2] and humans.[3] This is a result of noncreatinine chromagens that are measured as creatinine via the Jaffe reaction.[4] A falsely high serum creatinine concentration in the denominator of the clearance calculation results in an underestimation of GFR. Exogenous $Cl_{creatinine}$ circumvents this problem by minimizing the contribution of noncreatinine chromagens to the measured serum creatinine concentration by dilution.

Exogenous $Cl_{creatinine}$ can be used to approximate values for GFR that correlate with those of other methods.[1,5] Creatinine may be injected IV as a bolus or as

Received October 15, 2008.
Accepted February 9, 2009.
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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 $Cl_{creatinine}$ has been safely evaluated via SC injection in dogs[6] and healthy horse foals.[7] Although theoretically accurate, methods for determining $Cl_{creatinine}$ 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.[8]

The $Cl_{iohexol}$ has been used to estimate GFR in humans,[3,8–12] dogs,[6,13–20] cats,[16,21] pigs,[22] sheep,[23] and foals.[7] It is a safe and easy method that yields reproducible estimates of GFR, when compared with use of the inulin clearance and $Cl_{creatinine}$ techniques. In clinically normal foals, GFR determined by use of $Cl_{iohexol}$ agrees with GFR determined by use of exogenous $Cl_{creatinine}$.[7] Results of that study[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 $Cl_{iohexol}$ would yield estimates of GFR in horses similar to those determined by use of plasma $Cl_{creatinine}$.

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[a] 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[b]/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 $Cl_{iohexol}$—Iohexol[c] (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 and discarded. Another 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 × *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[d] for analysis.

Iohexol concentration in serum samples was determined via high-performance liquid chromatography by use of a method described in 1 study[24] as modified in another study.[14] Equipment included a separations module[e] with a dual-absorbance detector[f] at 254 nm and a 125 × 4.6-mm, 5-µm octadecylsilane column.[g] The detection limit was 5 mg of iohexol iodine/mL, and the limit of quantification in serum was 15 mg of iohexol/L.

Plasma $Cl_{creatinine}$—Aseptic techniques were used to prepare a creatinine solution[h] 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 35% of the creatinine dose was injected SC in the axillary, pectoral, and caudal cervical areas. Immediately after the remaining 35% 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[i] via a kinetic modification of the Jaffe method.[25] The $Cl_{creatinine}$ was calculated for each time interval by use of the following equation:

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

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 calculated to describe the data. Data were analyzed by use of nonlinear least squares regression analysis with equal weighting of the data via commercial software.[j] The following triexponential equation described the data for each horse:

$$Cs_t = (C_1 \times e^{-\lambda_1 t}) + (C_2 \times e^{-\lambda_2 t}) + (C_z \times e^{-\lambda_z t})$$

where Cs_t is the serum concentration at any time (t), C_1 and C_2 are concentration intercepts for the distribution phase, C_z is the concentration intercept for the postdistribution phase, λ_1 and λ_2 are slopes of the distribution phase curve, and λ_z 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 = $(C_1/\lambda_1) + (C_2/\lambda_2) + (C_z/\lambda_z)$. 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[j] on a personal computer. The $Cl_{iohexol}$ and $Cl_{creatinine}$ 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.[26] 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 $Cl_{3\text{-}4hours}$ was derived by use of errors in variables regression.

Results

Plasma $Cl_{creatinine}$—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 $Cl_{creatinine}$ 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

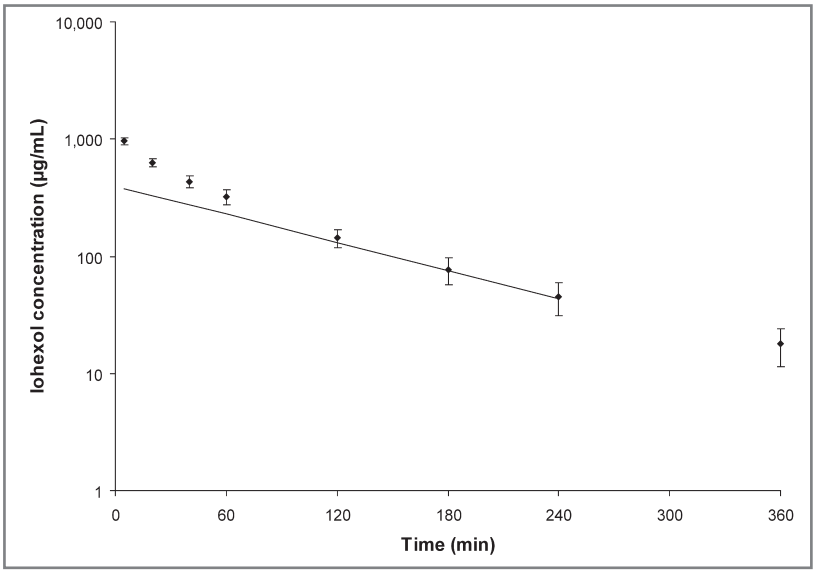

Figure 1—Mean ± SD semilogarithmic serum concentration of iohexol versus time in 10 horses after IV administration of a single dose (150 mg/kg). Time 0 corresponds to the time of completion of injection of the iohexol bolus and simultaneous SC injection of 65% of the creatinine dose. The solid line represents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

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

Variable	Median	Minimum	Maximum
C_1 (μg/mL)	471.7	306.0	543.2
C_2 (μg/mL)	541.4	136.5	754.4
C_z (μg/mL)	148.5	61.5	313.2
λ_1 (min^{-1})	0.127	0.025	0.236
λ_2 (min^{-1})	0.019	0.015	0.084
λ_z (min^{-1})	0.0062	0.0046	0.0115
AUC (μg/mL•h)	63,198.6	45,071.1	77,039.9
Cl_t (mL/min/kg)	2.37	1.95	3.33
Vc (L/kg)	0.1257	0.1064	0.1548
Vd_{area} (L/kg)	0.3875	0.2824	0.5197

The equation describing the 3-compartment model is as follows: $Cs_t = (C_1 \times e^{-\lambda_1 t}) + (C_2 \times e^{-\lambda_2 t}) + (C_z \times e^{-\lambda_z t})$, where Cs_t is the serum concentration at any time (t).

C_1 and C_2 = Concentration intercepts for the distribution phase. CL_t = Total clearance. C_z = Concentration intercept for the postdistribution phase. λ_1 and λ_2 = Slopes of the distribution phase curve. λ_z = Slope of the postdistribution phase curve. Vc = Volume of distribution of the central compartment. Vd_{area} = Volume of distribution during the terminal phase.

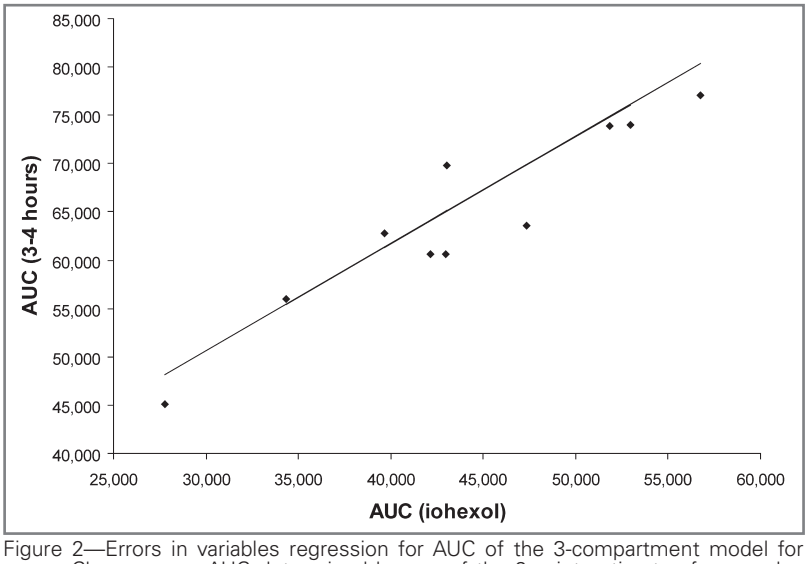

Figure 2—Errors in variables regression for AUC of the 3-compartment model for serum $Cl_{iohexol}$ versus AUC determined by use of the 2-point estimates for samples obtained at 3 and 4 hours. Each black diamond represents results for 1 horse, and the solid line represents the line of best fit of the data.

Akaike information criteria were used to determine that a 3-compartment model best described the data for 8 of 10 horses and a 2-compartment model best described the data for the other 2 horses. Because most of the data were best described with a 3-compartment model, this model was used for all horses. A semilogarithmic plot was made of mean iohexol concentration versus time (**Figure 1**). Pharmacokinetic variables were calculated for each model (**Table 1**). Mean $Cl_{iohexol}$ was 2.38 mL/min/kg (range, 1.95 to 3.33 mL/min/kg).

Values for 2-point estimates of serum $Cl_{iohexol}$—Terminal slopes of the elimination curve were calculated from each combination of the 3- and 4-hour, 4- and 6-hour, and 3- and 6-hour time points. Terminal slopes were used to estimate the concentration at time 0. The AUC was estimated as C_0/λ, where C_0 is the concentration at time 0, and λ 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: $AUC_{corrected} = (1.107716 \times AUC_{3\text{-}4hours}) + 15{,}731$, where $AUC_{corrected}$ is the corrected AUC, and $AUC_{3\text{-}4hours}$ 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 $Cl_{iohexol}$ and $Cl_{creatinine}$—Values for $Cl_{creatinine}$, $Cl_{iohexol}$ for the 3-compartment model, and $Cl_{3\text{-}4hours}$ were compared. For $Cl_{creatinine}$ versus $Cl_{iohexol}$, $Cl_{creatinine}$ versus $Cl_{3\text{-}4hours}$, and $Cl_{iohexol}$ versus $Cl_{3\text{-}4hours}$, the mean of the 2 methods was plotted against the difference between the 2 methods as described by Bland and Altman[26] (**Figure 3**). A paired *t* test was performed between the means of each method. The $Cl_{creatinine}$ was significantly different from $Cl_{iohexol}$ and $Cl_{3\text{-}4hours}$ ($P = 0.01$ and $P = 0.02$, respectively). There was no significant ($P = 0.12$) difference between $Cl_{iohexol}$ and $Cl_{3\text{-}4hours}$.

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

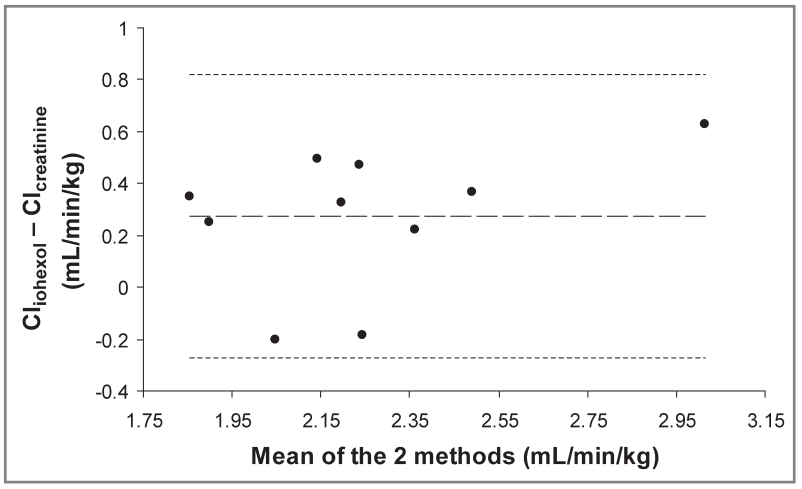

Figure 3—Bland-Altman plot to compare $Cl_{creatinine}$ versus $Cl_{iohexol}$ in 10 horses. Clearance values were calculated as the mean of the 2 methods and plotted against the difference between the 2 methods. Positive values indicate that $Cl_{iohexol}$ exceeded $Cl_{creatinine}$. Each black circle represents results for 1 horse. The dashed line represents the mean difference, and the dotted lines represent the limits of agreement.

glomerulus, is neither secreted nor reabsorbed by the kidneys, is not substantially bound to proteins or metabolized, and is not toxic.[8] Comparison of $Cl_{iohexol}$ with $Cl_{creatinine}$ should determine the usefulness of the former as an assessment of renal function. Clearance values determined in this study for both $Cl_{iohexol}$ and $Cl_{creatinine}$ were within reference intervals for GFR in adult horses determined by a variety of methods.[1,2,4,27–30] Serum $Cl_{iohexol}$ 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-fraught 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 $Cl_{creatinine}$, we suspect that serum $Cl_{iohexol}$ may be a more accurate measurement of GFR. To determine the validity of serum $Cl_{iohexol}$ 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, $Cl_{creatinine}$ 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 $Cl_{creatinine}$, 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 $Cl_{iohexol}$. Because results for the 3- and 4-hour sampling point were not significantly different from those of the other methods, and because this technique would be the easiest to perform clinically, this method of determining $Cl_{iohexol}$ may be an accurate and accessible technique for measuring GFR in horses. Although use of $Cl_{inohexol}$ in humans underestimates GFR when 4 or fewer sampling times are used,[3] analysis of our data revealed no difference between the estimation of GFR determined by use of 2 sampling points and by use of multiple sampling points. Future studies to evaluate use of $Cl_{iohexol}$ in horses with renal compromise may require different sampling times. Horses with GFR values within or greater than the reference range may require earlier sampling periods, whereas horses with compromised renal function that results in a low GFR may necessitate delayed sampling periods. Regardless, this study represented a logical first step in describing the use of $Cl_{iohexol}$ for estimation of GFR in horses.

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 $Cl_{iohexol}$ 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 $Cl_{iohexol}$ avoids all of these difficulties.

Other markers, including radiolabeled pharmaceuticals such as ^{99m}Tc-DTPA, have been used to estimate GFR.[27,32,33] Studies[27,32,33] 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 $Cl_{creatinine}$ was chosen as a clinical standard of measurement of GFR for comparison with $Cl_{iohexol}$ for this study. Although $Cl_{creatinine}$ 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 $Cl_{iohexol}$ 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 $Cl_{iohexol}$ in horses with evidence of renal dysfunction or failure.

a. Abbocath-T, Abbott Laboratories, Abbott Park, Ill.
b. Rompun, Bayer Corp, Shawnee Mission, Kan.
c. Omnipaque 350, Nycomed Amersham, Princeton, NJ.
d. Animal Health Diagnostic Laboratory, Michigan State University, East Lansing, Mich.
e. Alliance system 2695 separations module, Waters Corp, Milford, Mass.
f. 2487 dual-absorbance detector, Waters Corp, Milford, Mass.
g. Prodigy 5-µm ODS column, Phenomenex, Torrance, Calif.
h. Creatinine (C-4255), Sigma Chemical Co, St Louis, Mo.
i. Olympus AU400, Olympus, Dallas, Tex.
j. WinNonlin, version 1.5, Pharsight Corp, Mountainview, Calif.

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