Glomerular filtration rate in Holstein dairy cows estimated from a single blood sample using iodixanol

I. Murayama,* A. Miyano,† Y. Sasaki,† A. Kimura,†† S. Sato,†† and K. Furuhamat††¹

*Miyagi Prefectural Federation of Agricultural Mutual Aid Association, Miyagi 989-0731, Japan

†Cooperative Department of Veterinary Medicine, Iwate University, Morioka, Iwate 020-8550, Japan

††Unite Graduate School of Veterinary Science, Gifu University, Gifu 501-1193, Japan

ABSTRACT

The isotonic, nonionic, contrast medium iodixanol, as a test substance, was compared with the conventional glomerular filtration rate (GFR) tracer inulin to establish a simplified procedure for estimating the GFR in Holstein dairy cows. First, inulin and iodixanol were coadministered as a bolus intravenous injection to clinically healthy cows at 30 mg/kg and 10 mg of I/kg of body weight, respectively, followed by blood collection for multisample strategies. Serum iodixanol and inulin concentrations were separately determined by using HPLC and colorimetry, respectively, and blood urea nitrogen and creatinine concentrations in sera were measured. In the multisample method, the GFR values estimated by iodixanol were consistent with those estimated by inulin. No effects of body weight, age, or parity on GFR estimates were noted with either protocol used. No difference was observed between the GFR values obtained from nonlactating and lactating cows, suggesting that no transfer of iodixanol to milk occurred. An equation for calculating the GFR in the single-sample method was derived from the injected dose, sampling time, serum concentration, and estimated volume of distribution based on data from the multisample method in clinically healthy cows and cows with reduced renal function. The GFR values estimated by the single-sample method were in good agreement with those calculated by using the multisample method. These results demonstrate that the single-sample method using iodixanol can be applied as an alternative procedure for screening GFR in dairy cows.

Key words: glomerular filtration rate, Holstein dairy cow, iodixanol, serum clearance

INTRODUCTION

Urinary inulin clearance for estimating the glomerular filtration rate (GFR) is the most accepted index for kidney function assessment in humans (Imai et al., 2007) and small animals (Von Hendy-Willson and Pressler, 2011). However, the traditional method using inulin is labor intensive and time consuming because it requires accurately timed blood and urine samplings, and requires a long time to prepare the injectable solution because of its extremely low solubility. Use of radiolabeled compounds requires specific equipment and considerations that are uncommon in veterinary practice. Alternatively, endogenous creatinine clearance has been shown to have some advantages over inulin but is affected by other factors including active tubular secretion (Bauer et al., 1982; Darling and Morris, 1991). The nonionic, monomeric x-ray contrast medium iohexol as a GFR tracer has been used extensively in cats and dogs (Moe and Heiene, 1995; Brown et al., 1996; Von Hendy-Willson and Pressler, 2011), but concern with its use exists regarding impaired renal function (Miyamoto, 2001). Thus, efforts to find more reliable and less complicated tracers and approaches for assessing GFR are ongoing.

In cattle, only a few studies have reported GFR measurements to date (Anderson and Mixner 1960; Mercer et al., 1978). Here, we focused on the isotonic, nonionic, dimeric x-ray contrast medium iodixanol as a test tracer for estimating GFR in Holstein dairy cows. Iodixanol showed significantly fewer nephrotoxic effects in high-risk patients undergoing arteriography than did low-osmolality contrast media, including iohexol, in a randomized, double-blind, prospective, multicenter study (Karlsberg et al., 2010). Chemically, iodixanol has low osmolality and twice the iodine content per molecule of iohexol, and is assumed to show the same action as half the whole-body exposure dose (Kishimoto et al., 2008). According to a pharmacokinetic study using radiolabeled iodixanol in rats (Heglund et al., 1995), minimal fecal (0.9% by 24 h later) excretion was noted at a lower dose (0.2 g of I/kg), and the interspecies difference compared with monkeys was subtle.

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¹Corresponding author: furuhamat@iwate-u.ac.jp

Previously, Groth and Aasted (1981) devised a formula derived from a simple 1-compartment model combined with the volume of distribution (**Vd**) and optimum time for taking plasma using a specific tracer and accurately determined the GFR. Based on this information, the concentration of the GFR tracer in a single plasma sample taken a few hours after its injection was reported to be correlated with renal clearance (Jacobsson, 1983). More recently, we reported that the GFR in healthy calves estimated from a single blood sample using iodixanol (Imai et al., 2012) was in good agreement with historical reference data based on various tracers. Furthermore, serum creatinine concentrations became elevated when the GFR decreased to approximately 60% of the reference value in calves, as was also the case with rats (Katayama et al., 2010) and rabbits (Michigoshi et al., 2012).

The aim of the present study was to develop a simplified procedure for estimating GFR in Holstein dairy cows based on Jacobsson's formula (1983) using a single serum iodixanol concentration. This procedure is essentially that used for calves (Imai et al., 2012) but scaled up for dairy cows.

MATERIALS AND METHODS

Study Design

To elucidate whether the single-sample formula (Jacobsson, 1983) could be applied in Holstein dairy cows, we first compared the GFR values by the conventional 3- or 4-sample method using iodixanol with those by inulin. Briefly, inulin and iodixanol were coadministered intravenously to identical healthy nonlactating cows, and the respective GFR values were calculated by multisample strategies. The effects of BW, age, parity time, and milk yield on GFR estimates were examined. Next, by substituting the GFR values, the dose of iodixanol injected, and the single serum iodixanol concentration obtained at a certain sampling point in the multisample method into Jacobsson's (1983) formula, we calculated the estimated Vd value by solving this equation using the classic Newton method (Smale, 1985; Varona, 2002). Then, we examined the relationship between the estimated Vd value and serum iodixanol concentration by a scatter plot, and sought a new equation for obtaining the estimated Vd value from the serum iodixanol concentration at a single point. In the single-sample method, we estimated the GFR by substituting the dose injected, estimated Vd value, sampling time, and serum iodixanol concentration from each cow into Jacobsson's formula. Because the Vd value is dependent on the elimination kinetics of the tracer and animal size (Katayama et al., 2010), it is specific to individual ani-

mals. Finally, the relationship between GFR estimated by the multisample method and that from the single-sample method using iodixanol was determined using healthy cows and cows with reduced renal function.

Drugs

Iodixanol (Visipaque 320; 320 mg of I/mL, 290 mOsm/kg of H₂O) and inulin (Inulead; 100 mg/mL) from chicory were purchased from Daiichi-Sankyo (Tokyo, Japan) and Fuji Yakuhin (Saitama, Japan), respectively. The units for the dose level of iodixanol are milligrams of iodine/kilogram of BW. All other chemicals and reagents were of the highest grade available from commercial sources, unless otherwise stated.

Animals

The study used nonlactating and lactating Holstein-Friesian (Holstein) dairy cows weighing 120 to 920 kg, 0.5 to 8.5 yr old, and reared in stanchion barns around Shiroishi (Miyagi, Japan) and Koiwai (Iwate, Japan) areas and the Veterinary Teaching Hospital at Iwate University (Morioka, Japan). The animals were declared healthy after physical, hematological, and urinary examinations. The cows were fed orchardgrass and timothy mixed hay with concentrate in pens provided by feed bins and were given water *ad libitum*. Cows with reduced renal function included 7 clinical cases showing increases in BUN (>30 mg/dL vs. reference values in our laboratory of <25 mg/dL) and serum creatinine (>1.2 mg/dL vs. reference value in our laboratory of <1.0 mg/dL), the causes of which were unknown. In the present protocol, cows reaching 60 mg/dL for BUN or 6.0 mg/dL for serum creatinine concentrations were excluded, because the retention time of iodixanol in blood was unknown under the disease conditions. All procedures were performed in accordance with the Guidelines for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science (1987) or approved by the Animal Experimental Ethics Committee of Iwate University (reference number A201027).

Approximate Dose and Blood Collection Times of Iodixanol for GFR Estimations by the Multisample Method

To select the appropriate dose to estimate GFR, iodixanol was administered intravenously at a dose of 5, 10, or 20 mg of I/kg of BW to the jugular vein of healthy nonlactating, nonpregnant cows (*n* = 3) according to a 3 × 3 Latin square design. Blood was collected 60, 90, 120, and 150 min later via the opposite jugular vein.

The interval between the respective kinetics (5, 10, and 20 mg of I/kg) was at least 5 d.

To determine the appropriate blood collection times, iodixanol was given intravenously at a fixed dose of 10 mg of I/kg to healthy nonlactating cows ($n = 6$), and blood (1 mL) was collected preadministration and at 5, 15, 30, 45, 60, 90, 120, 150, and 180 min later. The representative combination of the sampling times for GFR estimates during the excretory phase was as follows: (a) 60, 90, 120, and 150 min later; (b) 60, 90, and 120 min later; (c) 60, 90, and 150 min later; (d) 60, 120, and 150 min later; and (e) 90, 120, and 150 min later.

GFR Value Estimations by the Multisample Method Using Inulin

Inulin was administered intravenously at 30 mg/kg to the jugular vein of healthy nonlactating, nonpregnant cows ($n = 3$), and blood was collected preadministration and at 5, 15, 30, 45, 60, 90, 120, 150, and 180 min later via the opposite jugular vein. The dose and blood collection times of inulin were chosen on the basis of data from the calf study (Imai et al., 2012) and from the results of a preliminary trial.

Relationship Between GFR Values Estimated by Coadministration of Inulin and Iodixanol in the Multisample Strategy

To confirm a correlation between GFR values estimated by inulin and iodixanol, the agents were coadministered intravenously at 30 mg/kg and 10 mg of I/kg, respectively, to identical healthy, nonlactating, nonpregnant cows ($n = 14$) and to 1 cow with reduced renal function. Blood (2 mL) was withdrawn preadministration and 30, 60, 90, and 120 min later.

Effects of BW, Age, Parity Time, and Lactating Yield on GFR Estimated by the Multisample Method

To examine the effect of BW, age, parity time, and lactating yield on GFR values, iodixanol was administered intravenously at 10 mg of I/kg to nonlactating ($n = 6$ to 7) or lactating cows ($n = 7$), and blood was withdrawn preadministration and 60, 90, and 120 min later. Lactating cows were divided into 3 categories consisting of <30 , <40 , and ≥ 40 kg of milk per day. Body weight- and age-matched nonlactating cows served as the corresponding controls. Moreover, milk (1 mL) from lactating cows was collected 1, 2, or 24 h after iodixanol injection and measured with reversed-phase HPLC method as mentioned below.

Laboratory Tests

Serum iodixanol concentration was measured by using HPLC according to the procedure reported previously (Jacobsen et al., 1995) with minor modifications (Katayama et al., 2010). The detection limit in the serum iodixanol assay was 5.0 μg of I/mL. Validation studies revealed no significant difference between serum and plasma iodixanol concentrations, and the intra- and interassay CV in serum iodixanol determination were within acceptable values (5% or less). Serum inulin concentration was determined using a commercially available kit (Diacolor-Inulin, Toyobo, Osaka, Japan). The detection limit in the serum inulin assay was 20 $\mu\text{g}/\text{mL}$. Neither iodixanol nor inulin interfered with the assay system of the other in *in vitro* and *in vivo* studies. Concentrations of BUN and creatinine in sera were measured with an autoanalyzer (Toshiba Medical Systems, Tochigi, Japan).

Calculation of Clearance

Clearance calculation was based on the 1- or 2-compartment model. Briefly, the area under the serum iodixanol or inulin concentration versus time curve (AUC) was calculated by the linear trapezoidal rule with extrapolation using 3 to 9 blood-sample points. A clearance value (CI) was calculated from the following formula:

$$\text{CI} = \frac{\text{dose}}{\text{AUC}}, \quad [1]$$

where dose is the dosage level of iodixanol or inulin injected.

To estimate iodixanol clearance by the single-sample method, the Vd value of iodixanol in each cow was back-calculated by substituting CI values (GFR) and serum iodixanol concentrations (C_t) at 60, 90, or 120 min (t) obtained from the multisample method using iodixanol into the following formula of Jacobsson (1983):

$$\text{GFR} = \frac{1}{t/V_d + 0.016} \times \ln \frac{\text{dose}}{V_d \times C_t}. \quad [2]$$

The Jacobsson (1983) formula can be transformed by the Newton method (Smale, 1985; Varona, 2002), and b , a variable value, can be solved:

$$V_d = t \frac{\text{GFR}}{b}. \quad [3]$$

The Vd value obtained was then reconfirmed by using the “Goal-Seek” function of Microsoft Office Excel 2007 (Microsoft, Tokyo, Japan). To seek the estimated Vd in each animal, after the correlation between the Vd value and C_t was assessed with an exponential regression, an equation for calculating Vd was determined. The Vd obtained from calculation was regarded as the estimated Vd in this study.

Finally, the GFR value obtained by the single-sample method using iodixanol was measured by substituting the estimated Vd value, the dosage level (10 mg of I/kg), and serum iodixanol concentration (C_t) at 60 min (t) from each animal into the above formula (eq. [2]). The GFR is expressed in milliliters per minute per square meter based on the body surface area (Holt et al., 1968). The equation used for body surface area was $0.09 \times BW^{2/3}$.

Sample Size and Statistical Analysis

The profiles of dairy cows used in this investigation are summarized in Table 1. For the study on the dose selection and blood sampling times of iodixanol or inulin, 12 nonlactating, nonpregnant cows weighing 270 to 700 kg and 1 to 3 yr old were used. For the simultaneous treatment study of inulin and iodixanol, 14 healthy nonlactating, nonpregnant cows weighing 500 to 800 kg, 2 to 4 yr old, and 1 cow with reduced renal function were used. For the study on the effect of BW, age, parity time, and lactating yield on GFR, 55 healthy nonlactating, nonpregnant cows weighing 120 to 920 kg, 0.5 to 8.5 yr old, and 21 healthy lactating and pregnant cows (with <30, <40, and ≥ 40 kg of milk per day) weighing 450 to 850 kg, 1 to 6 yr old, were also used. Seven cows with reduced renal function weighed from 210 to 840 kg and were 1.5 to 4 yr old.

Quantitative data are expressed as the mean $\pm$ SD. Differences among more than 3 groups were compared using one-way ANOVA and Dunnett’s test. A *P*-value of <0.05 was considered statistically significant. Comparisons of the GFR values from the multisample

method using inulin with those using iodixanol, or the GFR values from the multisample method with those from the single-sample method using iodixanol were performed according to a standard recommendation for comparing analytical techniques based on Deming’s regression (Deming, 1964) and Bland and Altman bias presentation (Bland and Altman, 1986, 1999) using Prism 5 (GraphPad Software, San Diego, CA).

RESULTS

Disappearance of Iodixanol or Inulin from Serum

In healthy nonlactating, nonpregnant cows given iodixanol at 5, 10, or 20 mg of I/kg, mean concentrations in serum disappeared with a semilogarithmic linearity from 60 to 150 min later at 5 mg of I/kg or more. At 5 mg of I/kg, however, serum iodixanol concentration 120 min later was near the detection limit (Figure 1A).

After iodixanol was intravenously injected at a dose of 10 mg of I/kg, changes in serum iodixanol concentration became biphasic, indicating the distribution and excretory phases. Calculation of the GFR using the 1-compartment model (GFR in the 4 blood-sample points: 195 to 220 mL/min per m^2) gave rates 10 to 15% higher than those calculated using the 2-compartment model (165 to 190 mL/min per m^2). In the present study, we calculated the AUC with the 1-compartment model in consideration of the application in the bovine practice (Figure 1B).

No difference was seen between GFR values for 4 (207 ± 10 mL/min per m^2) versus 3 blood-sample points (mean GFR ranges: 180 to 260 mL/min per m^2) in the 1-compartment model with various combinations of sampling times (Figure 2). For subsequent investigations, therefore, a combination of iodixanol at 10 mg of I/kg with blood-sample times of 60, 90, and 120 min later was selected for the multisample method.

In healthy nonlactating, nonpregnant cows given inulin at 30 mg/kg, mean concentrations in serum disappeared with a semilogarithmic linearity from 30 to 90 min later, as was seen for iodixanol.

Table 1. Summarized profiles of Holstein dairy cows used in the respective studies

Item	Pregnant or nonpregnant	Range				
		n ¹	BW (kg)	Age (yr)	BUN (mg/dL)	Serum creatinine (mg/dL)
Serum appearance of iodixanol	Nonpregnant	9	270–700	1–3	10–25	0.4–1.0
Serum appearance of inulin	Nonpregnant	3	270–700	1–3	10–25	0.4–1.0
Coadministration of iodixanol and inulin	Nonpregnant	15 (1)	500–800	2–4	10–25	0.4–1.0
BW, age, and parity	Nonpregnant	55	120–920	0.5–8.5	10–25	0.4–1.0
Milk transfer	Pregnant	21	450–850	1–6	10–25	0.4–1.0
Clinical cases	Both	7 (1)	210–840	1.5–4	35–60	1.2–6.0

¹Parentheses indicate use of the same cow.

Figure 1. Disappearance curve of serum iodixanol in healthy cows using a semilogarithmic plot. (A) Mean disappearance curve of cows given iodixanol intravenously at 5 (closed diamonds), 10 (open circles), or 20 (closed triangles) mg of I/kg of BW ($n = 3$); (B) representative disappearance curve in a cow given iodixanol intravenously at 10 mg of I/kg.

Relationship Between GFR Values Estimated by Coadministration of Inulin and Iodixanol

When inulin and iodixanol were coadministered intravenously at 30 mg/kg and 10 mg of I/kg, respectively, to identical 14 healthy cows and 1 cow with reduced renal function, the GFR values in 14 of 15 cows were within the 95% agreement plots (Figure 3). No adverse clinical signs associated with simultaneous administration were observed throughout the study.

Effects of BW, Age, Parity Time, and Lactating Yield on GFR

No effects of BW, age, parity time, or lactating yield on GFR estimated by the multisample method using iodixanol were noted, although GFR in cows weighing 120 to 200 kg fluctuated largely with no statistically significant difference among the other groups (Figure 4). No differences in estimated Vd (50 to 200 mL/kg) were seen among the lactating cows with 3 categories of milk yields and nonlactating cows. Iodixanol was not detected in milk in any of the lactating cows tested. No marked effects of different environmental factors including breeding areas (Miyagi versus Iwate), season (April to October), or feeding on the GFR values were observed.

Relationship Between GFR Values Estimated by the Single-Sample Method Versus Multisample Method Using Iodixanol

The equation for calculating the estimated Vd value was determined using healthy nonlactating ($n = 81$) and lactating cows ($n = 21$) and cows with renal insufficiency ($n = 7$) by a scatter diagram (Figure 5) as follows: Estimated Vd = $381.76e^{-0.058C}$, where C is serum iodixanol concentration at 60 min. Serum iodixanol concentration at 60 min ($r = 0.83$, $P = 0.01$, $n = 109$) was chosen because it showed a relatively high coefficient between Vd values and serum iodixanol concentrations compared with that at 90 ($r = 0.78$) or 120

Figure 2. Box plot showing changes in glomerular filtration rate (GFR) measured by combinations of various sampling points after a bolus injection of iodixanol at 10 mg of I/kg: (a) 60, 90, 120, and 150 min later; (b) 60, 90, and 120 min later (gray box); (c) 60, 90, and 150 min later; (d) 60, 120, and 150 min later; and (e) 90, 120, and 150 min later. The horizontal bar though the box represents the median; upper and lower bars show maximum and minimum values, respectively ($n = 6$).

Figure 3. Bland and Altman plot of the differences between glomerular filtration rate (GFR) values in 14 healthy cows and 1 cow with reduced renal function estimated by the multisample method using inulin ($\text{GFR}_{\text{inulin_multi}}$) and iodixanol ($\text{GFR}_{\text{iodixanol_multi}}$). Mean bias (solid line): -5.3 . Upper and lower values represent 95% agreement plots: mean bias ± 61.6 (dotted lines).

($r = 0.70$) min. Under the conditions of this study, the estimated V_d ranged from 50 to 250 mL/kg and from 5 to 100 mL/kg in healthy cows and cows with reduced renal function, respectively (Figure 5). No residual iodixanol in serum at 24 h postinjection was seen in any of the cows with reduced renal function.

A correlation was noted between the GFR values estimated by the multisample and single-sample methods using iodixanol ($r = 0.96$, $P < 0.001$, $n = 109$; Figure 6A). On the basis of the Bland and Altman bias plot, approximately 98.2% (107/109) of samples were within the agreement plots, although 2 points were outliers (Figure 6B).

DISCUSSION

The GFR in rats (Katayama et al., 2010), rabbits (Michigoshi, et al., 2012), and calves (Imai et al., 2012) estimated by iodixanol can be used to diagnose renal failure at an early stage, to detect a surviving mass in the kidney, or to comprehend pharmacokinetic alterations in pharmaceuticals depending on GFR changes, based on its excretory phase from serum.

Figure 4. Box plots showing effects of BW (A), age (B), parity time (C), and lactating (milk) yield (D) on glomerular filtration rate (GFR) values estimated by the multisample method using iodixanol in healthy cows. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively, point markers indicate outliers, and asterisks show extreme outliers ($n = 6$ to 7).

Figure 5. Scatter plot in estimated volumes of distribution (V_d) and serum iodixanol concentrations (C) 60 min after a bolus iodixanol injection in healthy nonlactating and lactating cows and cows with reduced renal function ($n = 109$). $V_d = 381.76e^{-0.058C}$, $r = 0.83$ ($P < 0.01$).

In multisampling strategies with Holstein cows, appropriate iodixanol dose and blood collection times were first determined for GFR estimations. When iodixanol at 10 mg of I/kg was administered intravenously to healthy nonlactating, nonpregnant cows, a linear semi-logarithmic plot of serum iodixanol concentrations versus time demonstrated the suitability of using the 1-compartment model. Moreover, no significant difference was detected between the GFR values estimated from 4 versus 3 blood collection times (60, 90, and 120 min later). These kinetics were a simplification and applied only after an equilibration period compared with the 2-compartment model.

Although an iodixanol dose of 40 mg of I/kg was used in the previous study with calves weighing <120 kg (Imai et al., 2012), the 10 mg of I/kg dose was chosen for dairy cows weighing 120 to 920 kg to reduce the administration volume (0.031 mL/kg of BW) with minimum exposure of the whole body to iodixanol.

For inulin, the 30 mg/kg dose and blood sampling times (30, 60, and 90 min later) were determined in the same trials. When inulin and iodixanol were co-administered intravenously to identical nonlactating, nonpregnant cows, GFR values in 14 of 15 animals were within the Bland and Altman agreement plots (Figure 2). Because inulin has an inherent disadvantage as a tracer (it requires boiling immediately before use), the subsequent investigations were performed using iodixanol as the test tracer.

The reference GFR range based on body size area was much more stable than that based on BW in the calf study (Imai et al., 2012). However, GFR values calculated based on body size area tend to increase

with BW in calves weighing 37 to 90 kg (Wanner et al., 1981). Under our conditions, no effect of BW on GFR estimates was noted, although GFR in cows weighing 120 to 200 kg fluctuated markedly. These fluctuations may result, in part, from rapid ruminal growth and increased total body water content. Moreover, dynamic

Figure 6. Relationship between the glomerular filtration rate (GFR) values estimated by the multisample method (GFR iodixanol_{multi}) and the single-blood-sample method (GFR iodixanol_{single}) using iodixanol in healthy nonlactating and lactating cows and cows with reduced renal function ($n = 109$). (A) Scatter plot of GFR between the 2 methods. Deming's regression was $y = 1.03x - 8.36$, $r = 0.96$ ($P < 0.001$); (B) Bland and Altman plot of the differences between the 2 methods. Mean bias (solid line): -0.9 . Upper and lower values represent 95% agreement plots: mean bias ± 29.2 (dotted lines).

changes in renal function may occur during the postnatal period. No effects of age, parity time, or lactating yield on the GFR estimates were observed. Because no differences in the estimated Vd or GFR values were observed between the nonlactating and lactating cows (Figure 5), we inferred that no transfer of iodixanol to milk occurred. If iodixanol were transferred into milk, the GFR values would presumably increase because of the lowered AUC that would result from reduced serum iodixanol concentrations by augmented Vd values. Taken together, our results indicate that the GFR measurement using iodixanol should apply extensively to dairy cows in various experimental and clinical settings.

Next, we focused on Jacobsson's formula (Jacobsson, 1983) for estimating the GFR with one sample, in which the Vd value is known and the accuracy in the Vd value determines the accuracy in the method (Groth and Aasted, 1981). The formula combined with the Vd value and optimum time gives an accurate GFR (Jacobsson, 1983). In our work, the estimated Vd values of individual cows were determined by substituting the GFR value obtained from the multisample method, the injected dose, and serum iodixanol concentration at 60 min into Jacobsson's formula, and completely solving by the Newton method (Smale, 1985; Varona, 2002). We were unable to find any reports of estimated Vd of iodixanol in cattle. Generally, an agent possessing a Vd value <600 mL/kg is considered to be in the extracellular fluid (interstitial volume outside the vessels) (Dittmer, 1961). As the Vd of iodixanol was 200 mL/kg or less (except for one cow), it was thought to exist only in the bloodstream and the extracellular fluid, but not in the intracellular fluid, 120 min after a bolus injection. A correlation was observed between the GFR values obtained from the multisample and single-sample methods using iodixanol. Based on Bland and Altman bias presentation, the GFR values obtained were frequently within the agreement plots between the 2 methods (Figure 6B). These findings suggested that the single-sample method using iodixanol can be used to estimate the GFR in cows as an alternative to the multisample method.

Using the single-sample method with iodixanol, the basal reference GFR values (229.4 ± 42.0 mL/min per m^2 , $n = 81$) obtained in clinically healthy, nonlactating, nonpregnant cows closely resembled the reported GFR data (Anderson and Mixner, 1960; Mercer et al., 1978), although the study protocols and tracers used were very different.

Concerning residues of iodixanol, the pharmacologically active ingredient of iodixanol is iodine, which is not considered, under Japanese regulatory law, to be a

substance having potential to cause damage to human health (Japanese Ministry of Health, Labour and Welfare, 2006). Iodixanol has been reported to be rapidly excreted into urine without metabolic degradation, with no or very little protein binding, and a very short half-life in experimental animals (Heglund et al., 1995) and humans (Svaland et al., 1992). The dose of iodixanol used here (10 mg of I/kg) corresponds to about one-fiftieth the human clinical dose (32 g of I/60 kg of BW) as a contrast medium for angiography, suggesting extremely low exposure to the entire body. At 1, 2, or 24 h after the bolus injection, no iodixanol (below the quantification limit) was observed in milk or sera, even in lactating cows and cows with reduced renal function. Moreover, nonionic iodine is seldom absorbed from the gastrointestinal tract (Heglund et al., 1995), unlike ionic iodine. Therefore, residual iodixanol in milk and meat of dairy cows may be ignored, although additional studies using sensitive analytical equipment are required.

Although the GFR calculated from the estimated Vd value is based on many assumptions to predict the true GFR, the equation derived from Jacobsson's formula should apply in dairy cows as well as rats (Katayama et al., 2010), rabbits (Michigoshi et al., 2012), and calves (Imai et al., 2012), as reported previously. However, because the Vd may be affected by different physiological (e.g., obesity) or disease (dehydration or ascites) conditions, further studies are necessary to collect cumulative background data, including GFR data for beef cows and cows with various types of renal failure.

CONCLUSIONS

The single-sample method based on Jacobsson's (1983) formula has some advantages, such as reduced multisampling stress and repeated application to identical cows. Moreover, fewer analytical procedures are required and exact timing of blood collection is unnecessary. This simplified method using iodixanol provides a practicable, ethical alternative for estimating GFR in Holstein dairy cows.

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REFERENCES

- Anderson, R. R., and J. P. Mixner. 1960. Inulin renal clearance in dairy cattle. *J. Dairy Sci.* 43:1476–1479.
- Bauer, J. H., C. S. Brooks, and R. N. Burch. 1982. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. *Am. J. Kidney Dis.* 2:337–346.
- Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1(8476):307–310.
- Bland, J. M., and D. G. Altman. 1999. Measuring agreement in method comparison studies. *Stat. Methods Med. Res.* 8:135–160.
- Brown, S. A., D. R. Finco, F. D. Boudinot, J. Wright, S. L. Taver, and T. Cooper. 1996. Evaluation of a single injection method, using iohexol, for estimating glomerular filtration rate in cats and dogs. *Am. J. Vet. Res.* 57:105–110.
- Darling, I. M., and M. E. Morris. 1991. Evaluation of “true” creatinine clearance in rats reveals extensive renal secretion. *Pharm. Res.* 8:1318–1322.
- Deming, W. E. 1964. *Statistical Adjustment of Data*. 1st ed. Dover Publication Inc., Mineola, NY.
- Dittmer, D. 1961. *Blood and Other Body Fluid*. Federation of American Societies for Experimental Biology, Washington, DC.
- Groth, S., and M. Aasted. 1981. ^{51}Cr -EDTA clearance determined by one plasma sample. *Clin. Physiol.* 1:417–425.
- Heglund, I. F., Å. A. Michelet, W. F. Blazak, K. Furuhashi, and E. Holtz. 1995. Preclinical pharmacokinetics and general toxicity of iodixanol. *Acta Radiol. Suppl.* 399:69–82.
- Holt, J. P., E. A. Rhode, and H. Kines. 1968. Ventricular volumes and body weight in mammals. *Am. J. Physiol.* 215:704–715.
- Imai, E., M. Horio, K. Nitta, K. Yamagata, K. Iseki, S. Hata, N. Ura, Y. Kiyohara, H. Hirakata, T. Watanabe, T. Moriyama, Y. Ando, D. Inaguma, I. Narita, H. Iso, K. Wakai, Y. Yasuda, Y. Tsukamoto, S. Ito, H. Makino, A. Hishida, and S. Matsuo. 2007. Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. *Clin. Exp. Nephrol.* 11:41–50.
- Imai, K., N. Yamagishi, N. Okura, T. Fukuda, T. Hirata, K. Okada, S. Sato, and K. Furuhashi. 2012. Estimation of glomerular filtration rate in calves using the contrast medium iodixanol. *Vet. J.* 193:174–179.
- Jacobsen, P. B., L. Blindheim, and T. Skotland. 1995. Bioanalytical methods for iodixanol and their application to studies on metabolism and protein binding. *Acta Radiol. Suppl.* 399:61–66.
- Jacobsson, L. 1983. A method for the calculation of renal clearance based on a single plasma sample. *Clin. Physiol.* 3:297–305.
- Japanese Association for Laboratory Animal Science. 1987. Guidelines for animal experimentation. *Exp. Anim.* 3:285–288.
- Japanese Ministry of Health. Labour and Welfare. 2006. Positive list system for agricultural chemical residues in foods. Notification No. 498. Ministry of Health, Labour and Welfare (MHLW): The provision of Paragraph 3, Article 11 of the Food Sanitation Law, as substances having no potential to cause damage to human health that the provision requires the Minister to specify. <http://www.mhlw.go.jp/english/topics/foodsafety/positivelist060228/>.
- Karlsberg, R. P., S. Y. Dohad, and R. Sheng. 2010. Contrast-induced acute kidney injury (CI-AKI) following intra-arterial administration of iodinated contrast media. *J. Nephrol.* 23:658–666.
- Katayama, R., N. Yamaguchi, T. Yamashita, S. Watanabe, H. Satoh, N. Yamagishi, and K. Furuhashi. 2010. Calculation of glomerular filtration rate in conscious rats by the use of a bolus injection of iodixanol and a single blood sample. *J. Pharmacol. Toxicol. Methods* 61:59–64.
- Kishimoto, M., K. Yamada, R. Tsuneda, J. Shimizu, T. Iwasaki, and Y. Miyake. 2008. Effect of contrast media formulation on computed tomography angiographic contrast enhancement. *Vet. Radiol. Ultrasound* 49:233–237.
- Mercer, H. D., L. B. Willett, F. L. Schandbacher, P. D. Moorhead, and T. E. Powers. 1978. Use of the double-isotope, single-injection method for estimating renal function in normal and polybrominated biphenyl-exposed dairy cows. *Am. J. Vet. Res.* 39:1262–1268.
- Michigoshi, Y., R. Katayama, N. Yamagishi, M. Kato, J. Saito, H. Satoh, and K. Furuhashi. 2012. Estimation of glomerular filtration rate in rabbits by a single-sample method using iodixanol. *Lab. Anim.* 46:341–344.
- Miyamoto, K. 2001. Clinical application of plasma clearance of iohexol on feline patients. *J. Feline Med. Surg.* 3:143–147.
- Moe, L., and R. Heiene. 1995. Estimation of glomerular filtration rate in dogs with $^{99\text{m}}\text{Tc}$ -DTPA and iohexol. *Res. Vet. Sci.* 58:138–143.
- Smale, S. 1985. On the efficiency of algorithms of analysis. *Bull. Am. Math. Soc.* 13:87–121.
- Svaland, M. G., T. Haider, K. Langseth-Manrique, E. Andrew, and P. A. Hals. 1992. Human pharmacokinetics of iodixanol. *Invest. Radiol.* 27:130–133.
- Varona, J. L. 2002. Graphic and numerical comparison between iterative methods. *Math. Intell.* 24:37–46.
- Von Hendy-Willson, V. E., and B. M. Pressler. 2011. An overview of glomerular filtration rate testing in dogs and cats. *Vet. J.* 188:156–165.
- Wanner, M., G. Ziv, J. Nicolet, U. P. Noelpp, and H. Roesler. 1981. Experiments with the double isotope single-injection method for determining glomerular filtration rate and effective renal plasma flow in veal calves. *Res. Vet. Sci.* 30:239–240.