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*Article*

# A Simple Method to Measure Renal Function in Swine by the Plasma Clearance of Iohexol

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**Abstract:** There is no simple method to measure glomerular filtration rate (GFR) in swine, an established model to study renal disease. We developed a protocol to measure GFR in conscious swine with the plasma clearance of iohexol. We used two groups: testing and validation, of 8 animals each. Ten milliliters of iohexol (6.47 g) were injected by the marginal auricular vein and blood samples (3 ml) were collected from the orbital sinus at different points after injection. GFR was determined considering two models: two-compartments (CL2: all samples) and one-compartment (CL1: the last six samples). In the testing group, CL1 overestimated CL2 by ~30%: CL2=245±93 and CL1=308±123 ml/mn. This error was corrected by a first order polynomial quadratic equation to CL1, which was considered the simplified method: SM=-47.909+(1.176xCL1)–(0.00063968xCL1$^2$). SM showed narrow limits of agreement with CL2, and a concordance correlation of 0.97 and a total deviation index of 14.73%. Similar results were obtained for the validation group. This protocol is reliable, reproducible, can be performed in conscious animals, uses a single dose of the marker, and requires a reduced number of samples avoiding urine collection. Finally, it portends a significant improvement in animal-welfare conditions and handling necessities in experimental trials.

**Keywords:** renal function; iohexol plasma clearance; swine model

## 1. Introduction

Translational studies in animal models are essential to evaluate the pathogenesis of renal disease. Most of basic research and preclinical studies in renal pathophysiology, like in other disciplines, have been performed in mice or rats. Rodents need reduced space, are relatively inexpensive to maintain, easy to manage, have a short life cycle and, in the case of mice, are easily modified by genetic engineering [1].Thus, during the last decades rodents have been extensively used as models for human renal disease [2].

However, although being the election model for basic research, rodents ~~seldom~~ do not completely recapitulate human renal disease as it has been observed for diabetic nephropathy [3] and membranous glomerulonephritis [4] and hemolytic uremic syndrome [5] among others renal diseases. To be fully translational, models of choice require kidney structure and function similar to humans. This is not the case of rodents, which have kidneys with a single papilla, undivided medulla and cortex. By the other hand, humans and swine show similar features of kidney structure, function and physiology. [6-9]. The swine kidney is multipyramidal with a cortex and several different medullary structures; each medullary pyramid forms a separate papilla and their fusion results in

the formation of some compound papillae. Renal physiology is also very similar between swine and men [8], with comparable maximal urine concentration (1080 and 1160mOsmol/l, respectively), maximal urine-to-plasma osmolal ratio (3.7 and 4.0), glomerular filtration rate (130 and 126-175ml/min per 70 kg) and total renal blood flow (4 and 3.0-4.4ml/min per gram). Hence, the swine is currently recognized as an amenable model for renal pathology [10-11]. The incidence of chronic kidney disease (CKD) and end stage renal disease (ESRD) is increasing worldwide, [12]. Moreover, age standardized death rates increased by 9% for diabetes and 37% for CKD, while those of non-communicable diseases decreased by 18% [13]. These changes have several causes; one of them may be the lack of reliable animal models of CKD and diabetic nephropathy. CKD is characterized by a progressive loss of the glomerular filtration rate (GFR). In studies using animal models of renal disease, aimed at studying the pathogenesis and the prevention of renal damage by new drugs, a reliable measurement of GFR is crucial. [14].

GFR can be measured by the clearance of inulin, radioactively labeled markers such as $^{51}$Cr-EDTA and ($^{125}$I)iothalamate, $^{99}$mTc-DTPA or non-radioactive markers such as iohexol [15,16] and iothalamate. Some of these methods are not simple or practical. Inulin requires constant infusion and a bladder catheter, which makes necessary, in animals, the use of anesthesia which could alter GFR and therefore the results of the experiment. $^{51}$Cr-EDTA and $^{99}$mTc-DTPA have the limitations of using radioactive markers. Finally, iothalamate may be affected by the existence of tubular secretion [17,18]. The plasma clearance of iohexol has several advantages in clinical practice and research, as recently reviewed [15,16]. This method is simple, reliable and a safe alternative to evaluate GFR [15,16]. Among its main advantages, iohexol is unbound to proteins, metabolically inert, freely filtrated by the glomeruli, neither secreted nor metabolized by tubular cells, and with negligible extra-renal clearance [15,16,19]. Moreover, iohexol is safe in patients with chronic kidney disease since there are no relevant changes in renal hemodynamics after its administration [15,16, 20].

However, very few studies evaluated the reliability of these methods in swine. The use of iohexol was early evaluated in swine [21] and compared with $^{51}$Cr-EDTA [22]. Frennby in 1997 described plasma and renal clearance of iohexol and $^{51}$Cr-EDTA in 21 anesthetized swine. Nevertheless, urine collection and multiple blood sampling were needed. Also, animals were handled under anesthesia, which may affect GFR. Finally, a simplified approach was tested in this study, using a correction formula designed for humans, which lead to overestimation of true GFR. Thus, to the best of our knowledge, there is no simple and reliable method to measure GFR in swine, which limits the utility of swine as an animal model of renal disease.

The objective of this study was to develop a simple and reliable method to measure renal function in conscious swine with the plasma clearance of iohexol, reducing the number of blood samples, in order to improve animal-welfare during the procedures, in accordance with Russell and Burch's 3Rs model for animal research (refinement, reduction and replacement; Russell, 1995) [23]

## 2. Results

### *2.1 Iohexol plasma analysis*

Figure 1 shows an HPLC-UV chromatogram of an iohexol-free blood sample before (Figure 1-A), and 120 minutes after injection (Figure 1-B). Iohexol eluted from the chromatographic column as two peaks at 4.03 and 4.47 minutes, reflecting the isomers present in the pharmacologic preparation. Dimethyluric acid (DMU) eluted at 6.10 minutes. None of the 16 animals evaluated showed interfering peaks in iohexol-free samples.

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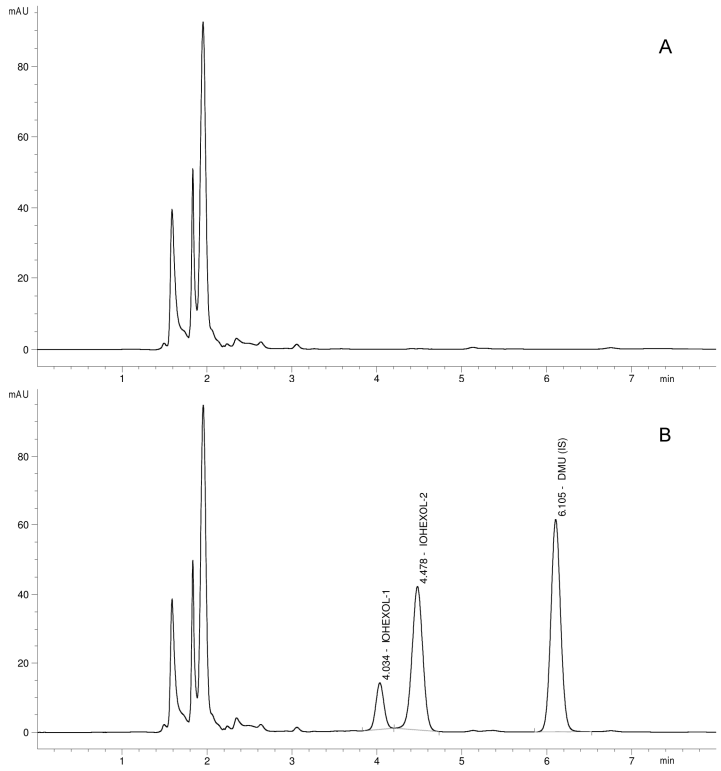


**Figure 1.** Representative chromatograms of swine plasma before (A) and after (B) intravenous iohexol (6.47 g) injection. Iohexol isomers and internal standard (IS) 1,3-Dimethyluric acid (DMU) were detected at 254 nm.

*2.2 Pharmacokinetic clearance profiles*

Figure 2 shows a two-compartment model for the iohexol plasma clearance. The first part of the curve, from 15 to 120 minute is curvilinear and corresponds to the distribution phase . The second part, from 120 to 420 minutes, is linear and corresponds to the elimination phase

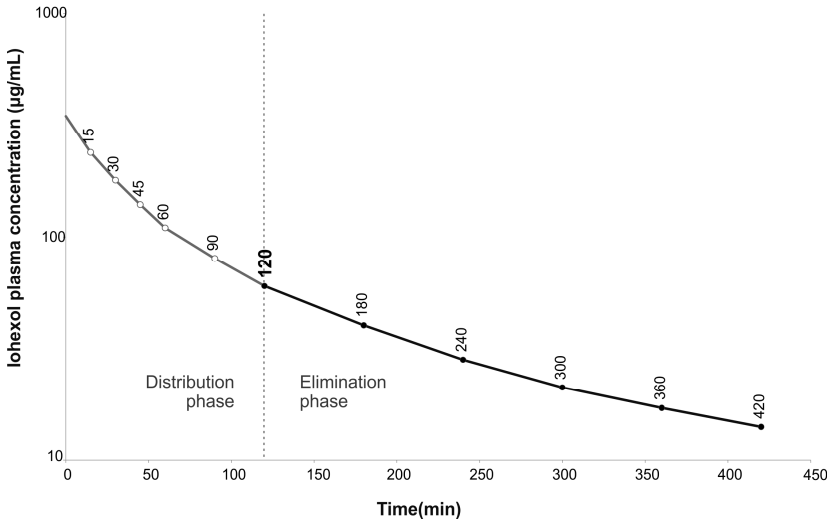


**Figure 2.** Pharmacokinetic profile of the iohexol plasma clearance by two-compartment model (CL2) in a representative swine. Sampling time points are indicated by diamonds (at 15, 30, 45, 60, 90 minutes) for the distribution phase and by dots (at 120, 180, 240, 300, 360 and 420 minutes) for the elimination phase. One-compartment model (CL1) considers only the elimination phase.

*2.3 Testing group*

Mean GFR values were 245±93 ml/min and 308±128 ml/min for CL2 and CL1, respectively (Table 1). For all cases, GFR was 20-30% greater when measured by CL1 than with CL2. The recalculation of CL1 by the Bröchner-Mortensen (BM) equation did not correct this difference. Moreover, this correction lead to a systematic underestimation of GFR values that averaged 23% (Table 1).

**T**able 1: Iohexol plasma clearance in one (CL1) and two-compartment (CL2) models in the testing and validation groups. SM = Simplified method. BM = Bröchner-Mortensen equation.*ml/min. **kg.

**TESTING GROUP**

| CASE | Weight ** | CL2 * | CL1* | SM* | SM-CL2 (%) | BM* | BM - CL2 (%) |
|---|---|---|---|---|---|---|---|
| 1 | 113 | 119.6 | 150.4 | 114.6 | -4.2 | 121.5 | 1.6 |
| 2 | 122 | 172.6 | 236.3 | 194.3 | 12.5 | 166.1 | -3.8 |
| 3 | 138 | 225.8 | 301.6 | 248.7 | 10.1 | 188.1 | -16.7 |
| 4 | 132 | 233.8 | 255.4 | 210.8 | -9.9 | 173.6 | -25.8 |
| 5 | 101 | 175.8 | 216.4 | 176.7 | 0.5 | 157.4 | -10.5 |
| 6 | 210 | 341.8 | 433.1 | 341.4 | -0.1 | 200.6 | -41.3 |
| 7 | 209 | 300.5 | 345.6 | 282.1 | -6.1 | 196.9 | -34.5 |
| 8 | 212 | 392.0 | 527.4 | 394.4 | 0.6 | 183.7 | -53.1 |
| mean±SD | 155 ± 47 | 245 ± 93 | 308 ± 123 | 245 ± 91 | | 190 ± 26 | |

**VALIDATION GROUP**

| | Weight ** | CL2 * | CL1* | SM* | SM - CL2 | BM* | BM - CL2 |
|---|---|---|---|---|---|---|---|
| 9 | 106 | 173.7 | 213.7 | 174.2 | 0.3 | 156.1 | -10.2 |
| 10 | 182 | 246.8 | 275.9 | 227.9 | -7.7 | 180.7 | -26.8 |
| 11 | 116 | 112.1 | 128.0 | 92.2 | -17.8 | 106.9 | -4.7 |
| 12 | 159 | 289.9 | 394.6 | 316.6 | 9.2 | 201.3 | -30.6 |
| 13 | 156 | 202.8 | 253.5 | 209.1 | 3.1 | 172.9 | -14.8 |
| 14 | 176 | 289.6 | 331.2 | 271.4 | -6.3 | 194.5 | -32.8 |
| 15 | 115 | 202.8 | 257.7 | 212.7 | 4.9 | 174.4 | -14.0 |
| 16 | 188 | 313.9 | 360.9 | 293.2 | -6.6 | 198.9 | -36.6 |
| mean±SD | 150 ± 33 | 229 ± 69 | 277 ± 85 | 225 ± 71 | | 181 ± 31 | |

*2.4 Correction formula*

The best formula to adjust CL1 to CL2 was the first order polynomial quadratic equation ($y_i=a+bx_i+cxi2$; R square: 0.97) (Table S1). Thus, the simplified method (SM) to calculate GFR in swine was SM= -47.909+(1.176xCL1)-(0.00063968x$CL1^2$). CL1 is the clearance obtained based on 1-compartment model and SM the recalculated true clearance with the simplified method. GFR in the testing group using the SM was 245.4±91.4 ml/min, which was similar to CL2: 245.3±92.8 ml/min. The error was < than 13% for all cases (Table 1). Individual GFR values are shown in Table 1. Finally, the cubic equation was not selected because the difference in GFR between CL2 and CL1 were higher than with first order polynomial quadratic formula.

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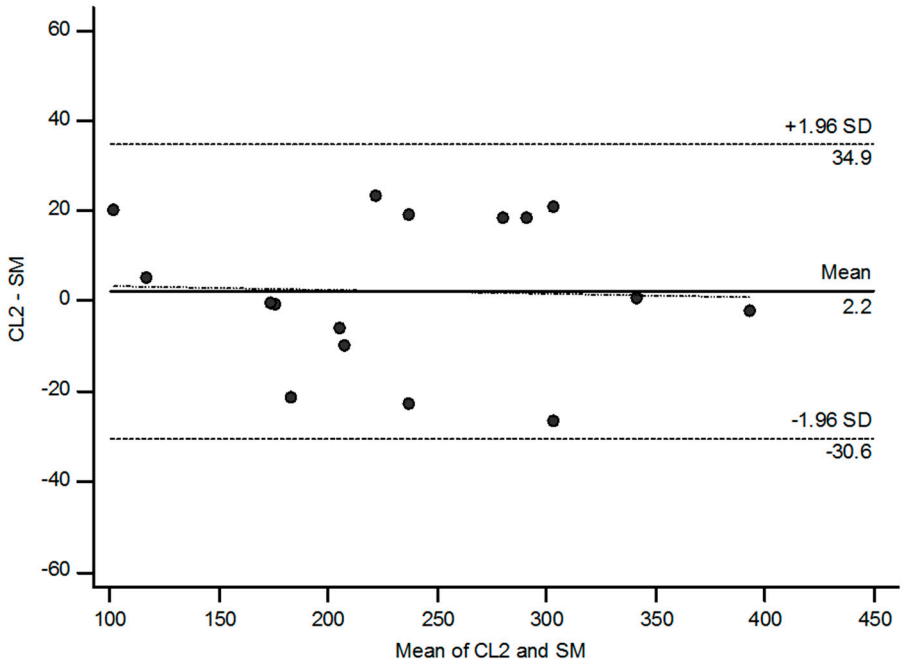
122 *2.5 Validation group*

123 Mean GFR values were 229±69 ml/min and 277±85 ml/min for CL2 and CL1, respectively (Table
124 1). The application of Bröchner-Mortensen equation to CL1 led to a 19% underestimation of GFR. On
125 the other hand, GFR assessed by SM was 225±71 ml/min, which is similar to CL2: 229±69 ml/min,
126 showing an error less than 10% for almost all animals (Table 1). Individual GFR values are shown in
127 Table 1.

128 *2.6 Analysis of agreement*

129 Bland and Altman plots (Figure 3) showed narrow limits of agreement (from -30.6 to 34.9
130 ml/min) and a mean difference of 2.1 ml/min between values measured with the simplified method
131 (SM) and the reference method (CL2), indicating good agreement.
132 Also, compared with the reference method (CL2), the simplified method (SM) had a concordance
133 correlation coefficient (CCC) of 0.97 (0.94, upper CI), reflecting high precision and accuracy. Also,
134 total deviation index (TDI) was 14.73% (20.62), which means that 90% of the GFR values showed an
135 error ranging from -14.7 to +14.7% when compared with the reference method. Finally, coverage
136 probability (CP) was 71 (54), which indicated that more than 29% of the GFR values had an error
137 range greater than ±10% of the method in plasma.

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139 **Figure 3.** Bland-Altman plots of the difference between the GFR values measured by the
140 reference (CL2) and the simplified method (SM) versus the mean of both. The straight and
141 the dashed lines indicate mean difference and 95% limits of agreement, respectively.

142 *2.7. Reproducibility study*

143 The mean absolute percentage error for the replicas in the overall group was 9.3 % (Table S2).

144 *2.8. Sensitivity analysis*

145 Comparable results were observed ~~between~~ when CL1 was calculated using the starting point
146 of the elimination phase at 120 or 180 minutes (data not shown).
147

### 2.9. Calibration and quality control standards

The differences between the experimental back-calculated concentrations of the calibration standards and the theoretical levels were within ± 5% for all the analyses. The deviations for the low and high quality controls were always lower than 7.5% (data not shown).

## 3. Discussion

The present study offers a new, simple, reliable and reproducible method to measure glomerular filtration rate (GFR) in swine with the plasma clearance of iohexol. The proposed method uses a specific correction formula applied to a one-compartment model pharmacokinetics. The procedure includes the following steps: i).- administration of 6.47 g of iohexol through an intravenous catheter placed at the marginal auricular vein; ii).- collection of six blood samples at 120, 180, 240, 300, 360 and 420 minutes after the iohexol injection; iii).- determination of plasma iohexol concentrations by HPLC-UV; iv).- calculation of the best fitting curve for these concentrations by a slope-intercept method; v).- calculation of iohexol plasma clearance as the ratio: dose/area under the curve; and vi).- correction of the obtained value by the proposed formula.

Our simplified method was performed in swine with normal GFR, paving the way for future studies in animals with reduced GFR. However, the applicability of the method is not expected to be dependent on the level of GFR. The mathematical approach will be the same for animals with normal, supranormal or reduced GFR, as it is in humans [15,16]. Such hypothesis is supported by previous studies of Brochner-Mortensen [24], who developed a formula in Caucasian which has been applied for any level of renal function in many studies.

This new proposed protocol has major advantages: it is performed in conscious animals, with no movement restriction, uses a single dose of the marker, requires a reduced number of blood samples (n= 6) and avoids urine collection. This represents a significant improvement of animal-welfare conditions and handling necessities in experimental trials that require the evaluation of GFR and eliminates the known influence of sedation or anesthesia on GFR [25-26].

For the selection of new methods in research with animal models, sample techniques must be reproducible and simple. Our method provides a simple and reliable approach for GFR measurement in swine, only using blood sample extractions. Urine sampling could be also used in swine, but implies catheterization of the urinary tract, which is technically difficult in these animals, and requires the use of anesthesia [27-29].

The clearance of inulin is considered the gold standard method for measuring GFR. The plasma clearance of iohexol showed a good correlation with the clearance of inulin [15-16]. In swine, Frenbby et al reported a difference of 4.0 ml/min per 10 kg weight between renal and plasma clearances of iohexol [21]. In this study, the last sample to calculate GFR was collected at 270 minutes, which may influence the results. The number and the timing for the last sample in multiple-sample approaches are fundamental to achieve acceptable precision and accuracy. The later the last sample is collected, the better the concordance between renal and plasma clearances of iohexol [16]. In our study the last sample was taken at 420 minutes, which may have led to a better agreement between our method and the urinary clearance of iohexol. In any case, urinary collection would make the whole procedure much more complex and difficult to perform.

The plasma clearance of iohexol has been originally described in 1984 in humans [30] and since then it has been frequently used in clinical research. Iohexol, is a stable molecule, which is freely filtrated through the glomeruli, it is not metabolized by tubular cells and is completely eliminated into the urine [31]. Moreover, the procedure is very safe, with few and minor side effects reported. Plasma clearance of iohexol is based on the disappearance curve of the marker, which typically fits to a two-compartment model with an initial rapid reduction (*distribution phase*) followed by a slow and linear decline in plasma concentrations (*elimination phase*). One-compartment models are focused only in the elimination phase which has the advantage of limiting the number of samples to estimate GFR which improves animal welfare, handling necessities and cost-efficiency. However, this approach does not take into account the early distribution phase which is a source of errors, making

necessary the use of a corrective formula [24]. A previous study in swine [21] used a formula developed in humans, the Bröchner-Mortensen equation, to correct the clearance derived from one compartment model [24]. However, this adjustment did not improve the performance of the simplified method. Brochner-Mortensen correction formula was developed in humans in whom the GFR values ranged from 0 to 120 ml/min [24]. The GFR values of the swine were about 2 times higher or even more and this may be the reason why the Brochner-Mortensen formula is not accurate in swine. In our study, we firstly evaluated a two-compartment model which was considered as the *reference method*. The clearance obtained using the values of the elimination phase lead to a 20-30% overestimation of true GFR measured by the *reference method.* Then, we developed a correction formula to adjust the clearance of the elimination phase to the reference method. This *simplified method* importantly reduced the error to less than 10%.

Finally, we tested the agreement between the values of GFR obtained by the simplified method and the 2 compartment model. The results of the Bland and Altmand test as well as the TDI and CCC showed good agreement, which allows the use of the proposed simple method for the evaluation of renal function in animal studies using swine.

## 4. Material and Methods

### *4.1 Ethics statement*

The study was performed according to the Spanish Policy for Animal Protection RD1201/05, which meets the European Union Directive 86/609 about the protection of animals used in research. The experiment was specifically assessed and approved (report CEEA 2012/012) by the INIA Committee of Ethics in Animal Research, which is the named Institutional Animal Care and Use Committee (IACUC) for the INIA. The sows were housed at the animal facilities of the INIA, which meets the local, national and European requirements for Scientific Procedure Establishments.

### *4.2. Experimental design*

We used two groups of animals (testing and validation) involving a total of 16 female adult Iberian swine (8 to 10 years old), formed by 8 animals each. The animals were conscious during all the experiment, restrained only for sampling during 3 to 5 minutes and free to move during the whole experiment. No sedation or anesthesia was used for the sampling procedure.

At 8:00 a.m after 6-8 hours of fasting, a single dose of 10 ml Omnipaque 300 (GE Healthcare) containing 6.47 g of iohexol was injected for 2 min through the marginal auricular vein of one ear. We selected a dose similar to that used in humans [15, 16]. After injection, blood samples (3 mL) were taken at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 minutes and collected in EDTA-treated tubes. This protocol was based on Frennby et al [21] with some modifications: i.e. samples were reduced from 16 extractions to 11 for the two compartment model, and 6 for the case of the one compartment model (see below). Blood samples were taken from the orbital sinus. In swine, phlebotomy can be difficult since there are few viable sites to draw blood; surface veins are small and the use of deep veins like cava and jugular is technically more complex, increases discomfort and risk for bleeding, especially in big animals. Blood collection from the orbital sinus is an established technique in veterinary described in 1969 and is minimally invasive and quick [32,33]. Also, animals exhibit little discomfort and return to their activities after the procedure is completed.

A blank (iohexol-free) blood sample was collected at time zero before the administration of the marker. Blood samples were immediately centrifuged at 2000g for 15 min and the plasma obtained was stored at -80 °C in the biobank.

### *4.3. Iohexol measurements*

Iohexol plasma concentrations were measured by HPLC-UV as previously reported [34]. Briefly, 200 µL of plasma were added 50 ml of internal standard (IS) 1,3-Dimethyluric acid (DMU) (500 mg/ml) and deproteinized with 750 µL of perchloric acid 5%. Samples were vortexed and centrifuged for 5 minutes at 12500 rpm. 5 µL aliquot of supernatant was chromatographed by a C18

reverse phase column (5mm, 150x4.6 mm, Advanced Chromatography Technologies LTD, Aberdeen, UK) using a HPLC system (Agilent Series 1260, Spain) equipped with a diode array detector set at 254 nm. Iohexol isomers were eluted by a mixture of deionized water/acetonitrile (96:4 by volume, adjusted to pH 2.5 with phosphoric acid) pumped at 1.0 ml/min flow rate. Calculation of iohexol concentrations were performed by using the height of the second isomer peak of iohexol to the IS peak (peak height ratio).

### *4.4. Calibration and quality control standards*

Internal calibration curves of iohexol were prepared for each set of samples. Working solution of iohexol (647 µg/ml) was prepared in deionized water and used for the calibration curve and quality control samples. A total of five concentrations of iohexol, namely 32.35; 64.7; 97.05; 129.4; and 161.75 µg/ml in drug-free plasma were used as calibrators. Two in-house quality control standards (QCs), containing iohexol at low (64,7 µg/ml**)** and high (129,4 µg/ml**)** concentrations were also prepared and used for assay validation. Aliquots of the calibrators, quality control samples and reference standard solutions were stored at −20 °C until use.

### *4.5. Pharmacokinetic analyses: one and two-compartment models*

a.- Two-compartment model (CL2): in the testing group, the concentrations of iohexol at 15, 30, 45, 60, 90, 120, 180, 240, 300, 360 and 420 minutes were fitted by nonlinear regression analysis to calculate the area under the curve (AUC). The iohexol plasma clearance was calculated as the ratio between dose of iohexol and AUC (dose/AUC).

b.- One-compartment model (CL1): in the testing group, only the elimination phase which starts at 120 minutes after the injection of the marker was considered. Then, the concentrations of iohexol at 120, 180, 240, 300, 360 and 420 minutes were fitted by a slope-intercept method to determine the area under the curve (AUC). The slope intercept method considers data only of the slow exponential and the fit is done by taking the natural logarithm of the plasma concentrations (Pi). The linear regression of ln(Pi) against the time (ti) is performed to determine the slope, -k, and the intercept, ln(P0). The AUC of the single exponential is given by AUC = (P0)/k. The iohexol plasma clearance was determined as the ratio dose/AUC.

### *4.6. Developing of a correction formula to simplify the method.*

GFR calculated by CL1 persistently overestimated true GFR assessed by CL2 (Table 1). The one-compartment model (CL1) underestimated the AUC because it did not consider the initial distribution phase of iohexol. Thus, a formula was needed to recalculate the true clearance. Based on a previous publication [21], we tested the Bröchner-Mortensen equation to adjust the values of CL1. Different equations were developed to recalculate CL1 using liner and non linear regression models. The best equation was selected based on the highest $R^2$. This equation was considered as the simplified method (SM) to measure GFR using the plasma clearance of iohexol. In the validation group, we calculated CL2 and CL1, and applied the SM as described above.

### *4.7. Reproducibility study*

The reproducibility of the plasma clearance of iohexol was determined in an extra group of 12 animals (2 to 3 years old) in which the method was performed two times, separated by 7 days. We calculated the absolute difference of the method in estimating GFR using mean absolute percentage error.

### *4.8. Sensitivity analysis*

To evaluate the validity of the starting point of the elimination phase at 120 minutes, we also calculated GFR using 180 minutes as starting point, and both GFR values were compared.

### 4.9. Pharmacokinetic Analysis

Results were expressed as mean±SD. The fit between CL1 and CL2 was evaluated with several regression models: linear, logarithmic, inverse, quadratic, cubic, compound, power, S-curve, exponential and logistic. All data were fitted by a nonlinear regression iterative program. The best equation was selected based on the higher R square and the lower differences between CL2 and CL1. The formula was applied to CL1 and this was considered the simplified method for the iohexol plasma clearance. Calculations and graphical representation were performed with SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, Il., USA).

### 4.10. Statistical analysis: tests of agreement

The agreement between CL2 and SM was assessed by the limits of agreement described by Bland and Altman [35] and the total deviation index (TDI), concordance correlation coefficient (CCC) and coverage probability (CP) as proposed by Lin et al. [36]. The limits of agreement are a simple graphic tool which describes the limits that include the majority of the differences between two measurements. The narrower these limits are, the better the agreement. CCC combines elements of accuracy and precision. Its scores range from 0 to 1 and a value > 0.90 reflects optimal concordance between measurements. TDI is a measure that captures a large proportion of data within a boundary for allowed differences between two measurements [36]. CP ranges from 0 to 1; it is a statistic that estimates whether a given TDI is less than a pre-specified percentage [37]. The ideal situation is to have a TDI <10%, meaning that 90% of the estimations fall within an error of ±10% from the gold standard. Finally, these statistics provide confidence intervals which allow generalization of the results.

For the Bland and Altman test we used the MedCalc statistical package, version 15.8. For the agreement analyses, we used the statistical package AGP (Agreement Program) v.1.0 (IGEKO, SP) available: at http://investigacion.chuc.es/2011-09-10-20-17-00/area-de-metodologia. The AGP is based on the R code originally developed by Lawrence Lin and YuYue [37]. The AGP was developed to simplify the use of the tool given in the R agreement package.

## 5. Conclusion

In conclusion, we have developed a *simplified method* to measure renal function in swine which is simple, reproducible and reliable, accurate and precise, requires a reduced number of blood samples and improves animal management and welfare. Moreover, this new method facilitates sequential measurements of renal function, which allows the assessment of changes in GFRover time. Finally, the proposed protocol is similar to the one used in clinical research in humans, which will facilitate translational studies.

## Supplementary Material

**Table S1. Regression models**. 10 linear and non-linear regression models were developed

| | Resume of the models | | | | | Regression coefficients | | | |
|---|---|---|---|---|---|---|---|---|---|
| Equation | R square | F-Snedecor | DF 1 | DF2 | *P*-Value | Intercept | Beta 1 | Beta 2 | Beta 3 |
| Linear | 0.96 | 141.7 | 1 | 6 | <0.001 | 17.885 | 0.737 | | |
| Logaritmic | 0.95 | 127.6 | 1 | 6 | <0.001 | -1035.718 | 226.259 | | |
| Inverse | 0.87 | 39.9 | 1 | 6 | 0.001 | 459.672 | -57505.551 | | |
| Quadratic | 0.97 | 77.1 | 2 | 5 | <0.001 | -47.909 | 1.176 | $-6.3968 \times 10^{-4}$ | |
| Cubic | 0.97 | 43.3 | 3 | 4 | 0.002 | 23.880 | 0.407 | 0.002 | $-2,493x 10^{-6}$ |
| Compound | 0.90 | 54.8 | 1 | 6 | <0.001 | 89.741 | 1.003 | | |
| Power | 0.96 | 148.9 | 1 | 6 | <0.001 | 0.960 | 0.967 | | |
| S-curve | 0.94 | 98.3 | 1 | 6 | <0.001 | 6.388 | -255.2 | | |

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| | Resume of the models | | | | | Regression coefficients | | | |
|---|---|---|---|---|---|---|---|---|---|
| Equation | R square | F-Snedecor | DF 1 | DF2 | *P*-Value | Intercept | Beta 1 | Beta 2 | Beta 3 |
| Exponential | 0.90 | 54.8 | 1 | 6 | <0.001 | 89.741 | 0.003 | | |
| Logistic | 0.90 | 54.8 | 1 | 6 | <0.001 | 0.011 | 0.997 | | |

**Table S2. Reproducibility study.** Iohexol plasma clearance for the Simplified method (SM) in two occasions in two occasions on two weeks apart in a group of 12 adult iberian swine. The precision (time-to-time variability) was evaluated as mean absolute percentage error (MAPE) of GFR (ml/min) for each case.

| SWINE ID | GFR REPLICA 1 | GFR REPLICA 2 | MAPE |
|---|---|---|---|
| 1 | 131.3 | 126.4 | 3.7 |
| 2 | 122.9 | 128.5 | 4.5 |
| 3 | 175.5 | 151.1 | 13.9 |
| 4 | 146.5 | 121.8 | 16.8 |
| 5 | 221.7 | 185.5 | 16.4 |
| 6 | 142.4 | 146.6 | 2.9 |
| 7 | 154.2 | 167.1 | 8.4 |
| 8 | 188.1 | 191.1 | 1.6 |
| 9 | 136.5 | 155.3 | 13.8 |
| 10 | 202.4 | 217.7 | 7.6 |
| 11 | 164.5 | 184.0 | 11.8 |
| 12 | 245.6 | 219.8 | 10.5 |
| Mean | 169.3 | 166.3 | 9.3 |

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