Validation of iohexol as an accurate and sensitive marker of renal function in rat models

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Introduction

The lack of tools for longitudinal assessment of renal function in animal models that are accurate, sensitive and simple to use hinders experimental research of CKD onset and novel interventions.

- Plasma creatinine concentration, the most widely used estimator of renal function, has limited accuracy and sensitivity due to endogenous function and tubular secretion[1].
- Urinary clearance of inulin, the gold standard measurement of GFR, is expensive and has significant methodological difficulties in animal models which are not feasible for repeated measurement.
- Estimating GFR via plasma clearance of exogenous substances is an alternative. Sturgeon *et al.* obtained equivalent estimations of GFR in rats via inulin urinary clearance and inulin plasma clearance[2].
- Iohexol is an inexpensive, low-osmolarity radio-contrast agent that has significant clinical relevance for plasma clearance[3].

Objectives

In a rat model of progressive CKD, the study aimed to:

#1: Longitudinally evaluate the agreement of iohexol plasma clearance to previously established inulin plasma clearance.

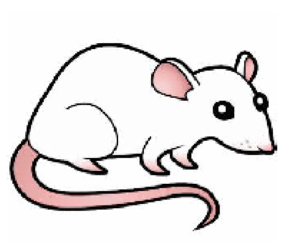
#2: Compare the sensitivity of iohexol plasma clearance to inulin plasma clearance and plasma creatinine concentration

#3: Investigate the impact of model simplification on the above parameters.

Methods

- Renal function assessment performed *weekly* in progressing adenine-induced CKD model (baseline + 5 weeks of CKD) in Sprague-Dawley rats (N=8, 400-475g)

Renal function assessment protocol:



Serial Tail Vein Injection of:
Iohexol "Omnipaque" (12 mgl/kg)
5% **FITC-Inulin** Solution (3 mL/kg)
Heparinized Saline (300 uL of 50 units/mL)

↓

12 saphenous blood samples (plasma) over 5 hrs.

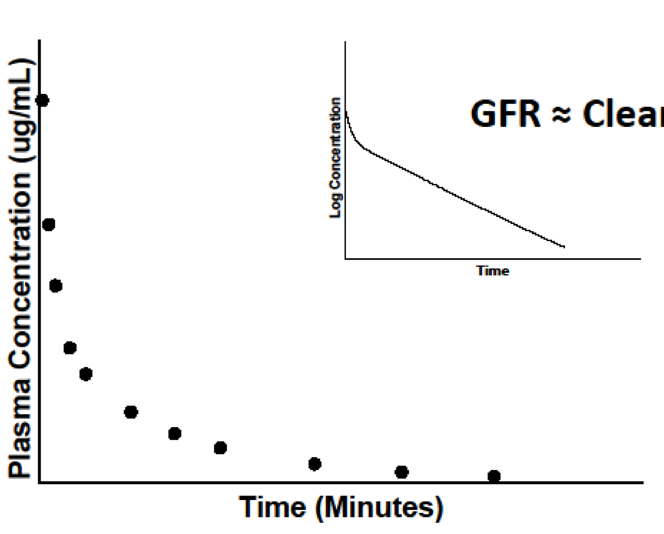
↓

Plasma measurement of:		
FITC-Inulin	→	fluorometry[4]
Iohexol	→	LC-MS[5]
Creatinine	→	Jaffey Method

↓

Pharmacokinetic Analysis

Plasma clearance curve for FITC-inulin + iohexol



$$\text{GFR} \approx \text{Clearance} = \frac{\text{Dose}}{\text{Area Under the Curve}}$$

Methods for Calculating Area Under the Curve:

A. Trapezoidal approximation (AUC)
B. 2-Compartment Model
} *Requires full clearance curve elucidation*
C. 1-Compartment Model – Simplified: requires *only 2+* samples

Summary & Conclusion:

Plasma iohexol clearance and plasma inulin clearance strongly correlate using all models (R^2=0.86-0.93), but most importantly, correlate well (R^2=0.93) with no measurable bias (0.01±0.27 mL/min) using a simplified 1-compartment model (Fig. 3) and measures significant renal impairment at the first week of CKD treatment.

The data supports a simplified protocol using 2-3 blood samples, following a tail vein injection of iohexol, fit to a 1-compartment model will be simple, accurate and sensitive to early dysfunction. This method is superior to creatinine and to methodologically complex urinary clearance or full curve elucidative plasma clearance.

Results

#1: Plasma clearance of inulin and iohexol have high-agreement using a 12-point model

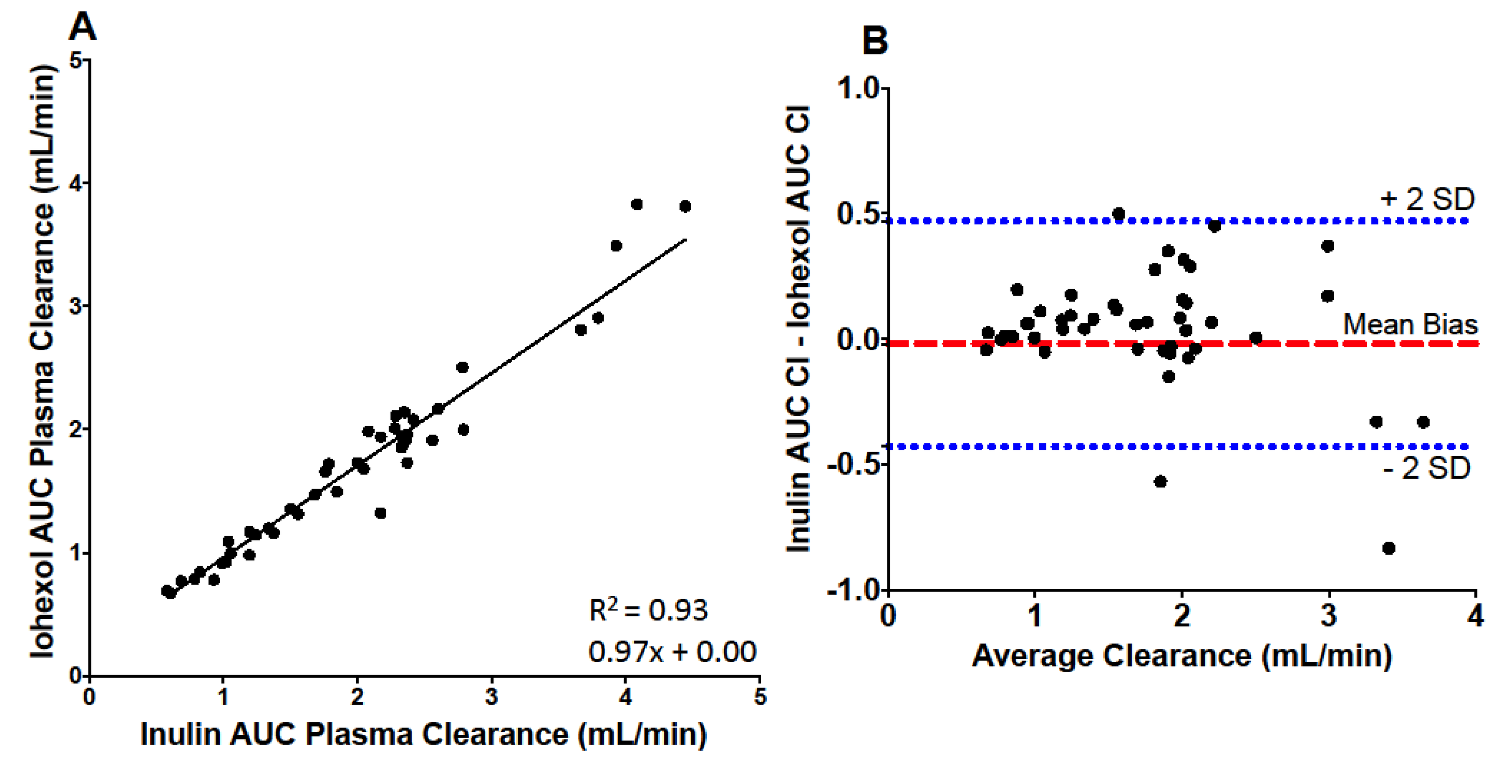


Figure 1: A. Linear regression of plasma clearance of inulin and iohexol using a trapezoidal approximation of 12 points. B. Bland-Altman plot of bias and precision. See Table 1 for values.

#2: Plasma clearance of Iohexol has equivalent sensitivity to inulin superior by 2 weeks to creatinine

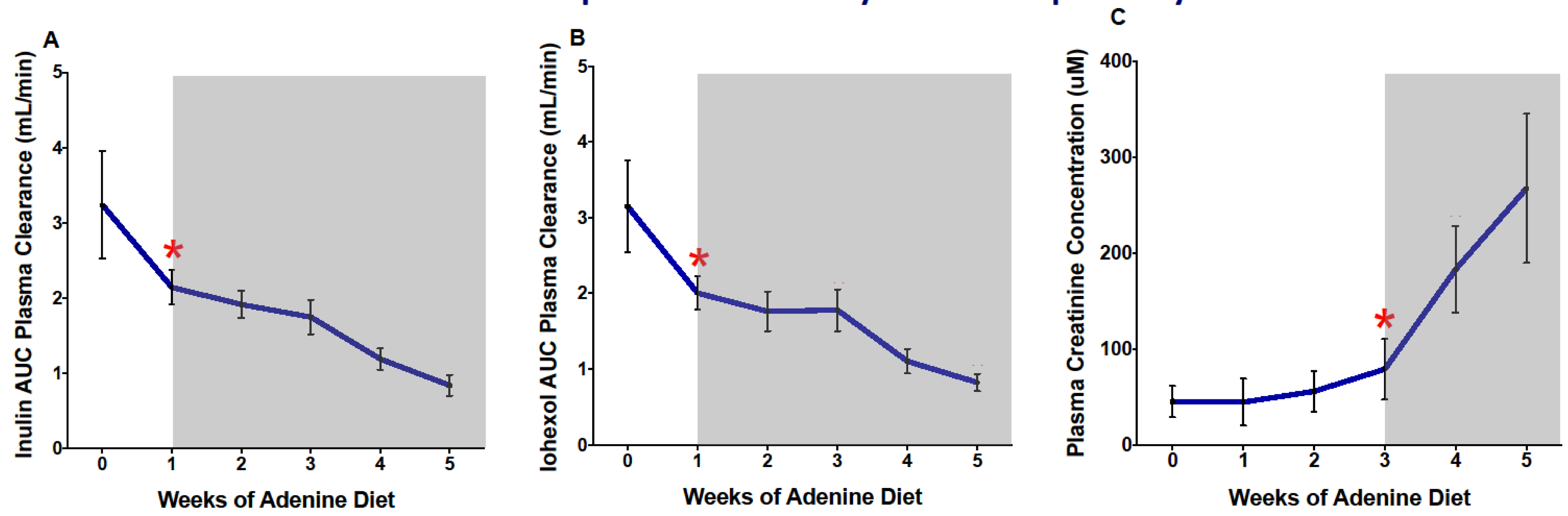


Figure 2: Plasma Clearance Values and Creatinine Concentrations by Week. Shaded area indicates a significant impairment of clearance (p<0.005) or increase in creatinine concentration (p<0.02).

#3: A simplified model of iohexol plasma clearance maintains sensitivity and agreement with inulin

Sensitivity: Iohexol 1-compartment clearance significantly decreased from baseline at week 1 of CKD (p=0.008)

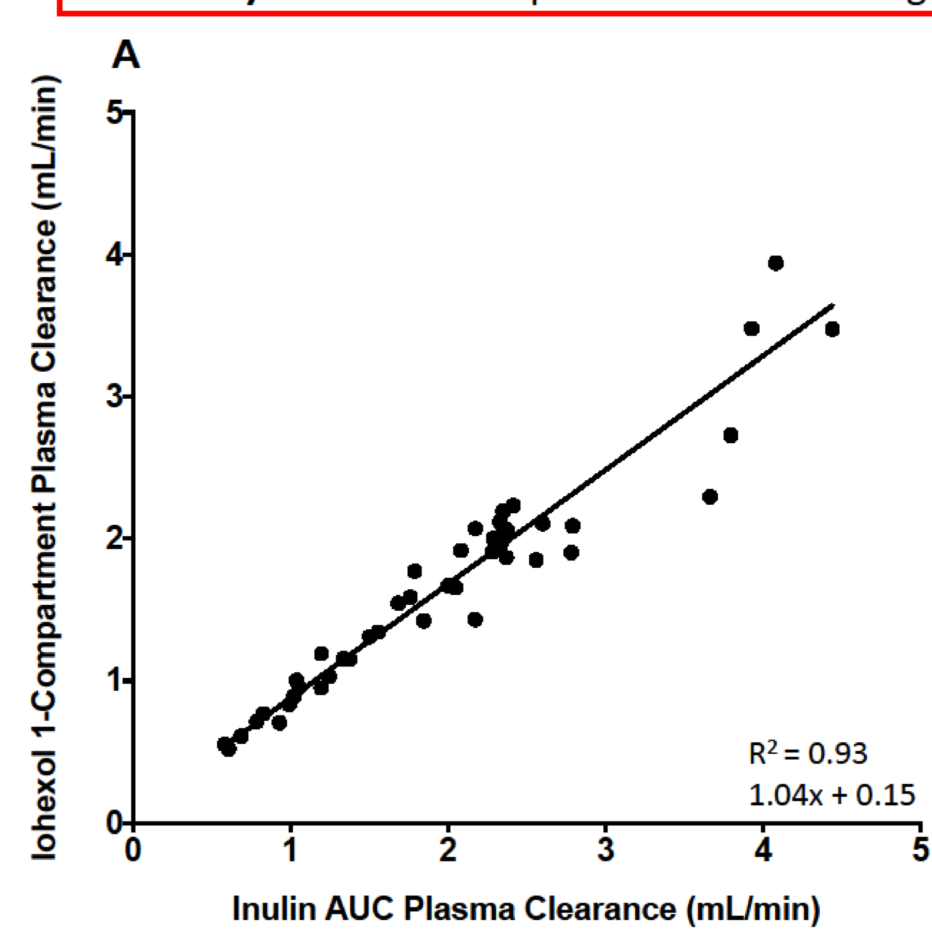


Figure 3: A. Linear regression of plasma clearance of inulin and iohexol using trapezoidal approximation of 12 points. B. Bland-Altman plot of bias and precision. See Table 1 for values.

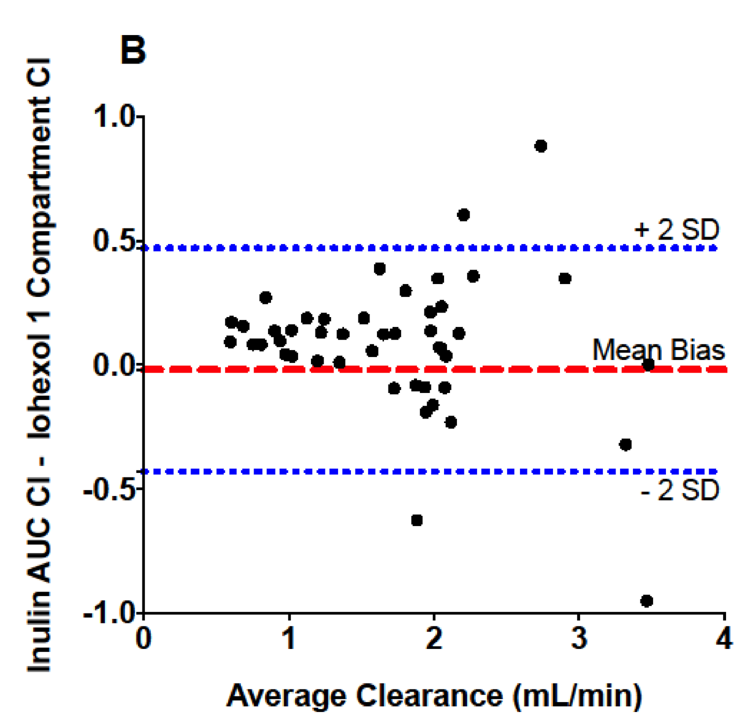


Table 1: Linear regression coefficients, bias, precision and accuracy of plasma clearance compared to AUC inulin plasma clearance as a reference method.

Comparative Clearance	R^2	Bias Precision	% Bias Precision	10% Accuracy
IHX – 2 Compartment	0.86	0.04 ± 0.37	6.0 ± 16.9	48.8
IHX - AUC	0.93	-0.02 ± 0.23	3.9 ± 10.9	74.4
IHX – 1 Compartment	0.93	0.01 ± 0.27	6.8 ± 13.3	58.1
INU – 1 Compartment	0.98	-0.79 ± 0.42	-22.1 ± 13.2	25.5
Creatinine	0.53			

References

1. Suckow, M. A., Weisbroth, S. H., & Franklin, C. L. (Eds.). (2005). *The laboratory rat*. Academic Press.
2. Sturgeon, C., Sam, A. D., & Law, W. R. (1998). Rapid determination of glomerular filtration rate by single-bolus inulin: a comparison of estimation analyses. *Journal of applied physiology*, *84*(6), 2154-2162.
3. Reischig T, Jindra P, Hes O, Svecova M, Klaboch J, Treska V. Valacyclovir prophylaxis versus preemptive valganciclovir therapy to prevent cytomegalovirus disease after renal transplantation. American Journal of Transplantation 2008; 8(1):69-77.
4. Qi, Zhonghua., Whitt, I., Mehta, A., Jin, J., Zhao, M., Harris, R. C., ... & Breyer, M. D. (2004). Serial determination of glomerular filtration rate in conscious mice using FITC-inulin clearance. *American Journal of Physiology-Renal Physiology*, *286*(3), F590-F596.
5. Annesley, T. M., & Clayton, L. T. (2009). Ultraperformance liquid chromatography–tandem mass spectrometry assay for iohexol in human serum. *Clinical chemistry*, *55*(6), 1196-1202.

Funding provided by:
CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada