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Modelling Acute Renal Failure using Blood and Breath Biomarkers in Rats

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Abstract: This paper compares three methods for estimating renal function, tested in rats. Acute Renal Failure (ARF) was induced via a 60-minute bilateral renal artery clamp in 8 Sprague-Dawley rats and renal function was monitored for 1 week post-surgery. A two-compartment model was developed for estimating renal function via a bolus injection of a radio-labelled inulin tracer, and was compared with the current gold standard plasma creatinine measurement, modified using the Cockcroft-Gault equation for rats. These two methods were compared with Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) monitoring of breath analytes. Determination of renal function via SIFT-MS is desirable since results are available non-invasively and in real time. Relative decreases in renal function show excellent correlation between methods, and indicate good promise for fast, non-invasive determination of renal function via breath testing.

Keywords: Renal Function, Breath Biomarkers, Integral Fitting Method, Model-based Approximation, Tracer Kinetics, Differential Equations.

1. INTRODUCTION

Current measurements of renal function rely on daily measurements of plasma creatinine. When most glomerular filtration function is lost, the diagnosis of kidney failure is delayed by up to 3 - 5 days in clinical situations. This results in unacceptable delays in instituting treatment. Consequently, there have been no improvements in mortality in the management of Acute Renal Failure (ARF) for over 50 years, despite the availability of many useful experimentally effective treatments.

Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) is an analytical technique for the real-time quantification of Volatile Organic Compounds (VOCs) in air or breath samples. By analyzing changes in VOC concentrations in breath over time, SIFT-MS can offer fast diagnosis of various conditions and diseases non-invasively and in real-time.

In this study, ARF is induced in 8 Sprague-Dawley rats. Renal function is monitored for 1 week following induction of ARF via plasma creatinine measurements, SIFT-MS breath sampling, and radio-labelled inulin clearance tests. The goal is to correlate changes in breath biomarkers with the current clinical and model-based gold standards.

1.1 Glomerular Filtration Rate (GFR)

Glomerular Filtration Rate (GFR) refers to the volume of fluid filtered from the glomerular capillaries into Bowman's Capsule of the kidney per unit time (Fig. 1), and is generally accepted as the best overall estimation of kidney function. Any substance that is freely filtered by the glomerulus and is not secreted or reabsorbed by the kidneys can be used to measure GFR.

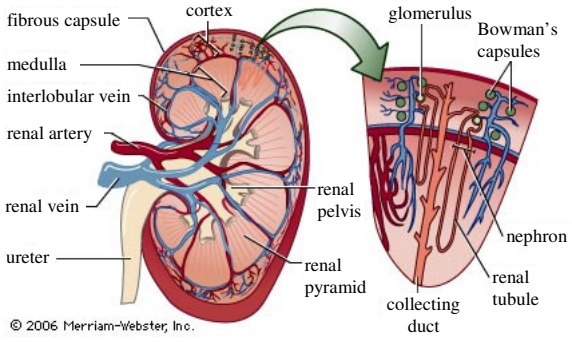

Fig. 1. Kidney Anatomy (Merriam-Webster Inc., 2006)

1.2 Exogenous Markers for measuring GFR

If an exogenous filtration marker, such as inulin, is used to measure GFR, it is injected into the plasma, and its clearance through the kidney can be measured using either constant infusion or bolus techniques (Van Acker, et al., 1995; Davison, et al., 1992; Sturgeon, et al., 1998). Using the constant infusion technique, the exogenous marker is injected into the plasma at a constant rate until a steady state is achieved. At this point, (1) is applied, where plasma and timed urine samples are collected to determine the marker concentration (*Urine Conc* and *Plasma Conc*, µmol/L), and the rate of urine flow (Urine Flow, mL/min).

$$GFR = \frac{Urine\ \ Conc \times Urine\ \ Flow}{Plasma\ \ Conc} \quad (1)$$

In practice, this technique has limitations, as it is difficult and slow to achieve a steady state. In contrast, bolus methods can be quickly carried out, and require only a single injection of the exogenous marker into the plasma, and serial blood sampling at defined intervals. Urine collection is not required. However, data analysis requires the application of a well defined mathematical model.

1.3 Endogenous Markers for measuring GFR

Measuring GFR via the clearance of an exogenous marker is time consuming, expensive, and thus not appropriate in most clinical settings. Hence, GFR is usually estimated using an endogenous marker, such as creatinine. Steady state serum creatinine is approximately inversely proportional to GFR. However, creatinine is not an ideal filtration marker because, in addition to being filtered by the glomerulus, it is also secreted by the proximal tubules, resulting in a clearance that exceeds GFR, particularly in ARF.

Since creatinine generation is determined mainly via muscle mass and dietary intake, tubular secretion of creatinine depends on factors such as age, sex, race, body size and muscle mass, and can vary substantially between individuals and over time, (Davison et al. 1992). Equations such as the Cockcroft-Gault and the MDRD equations have been developed to try to correct for these limitations (Cockcroft & Gault 1976). Additionally, extra-renal elimination of creatinine via intestinal bacteria can increase at low GFR, thus making the method misleading for those individuals outside the normal range of kidney function.

1.4 SIFT-MS Breath Sampling

SIFT-MS is a quantitative mass spectrometric method that utilizes the chemical ionization of positively charged precursor ions that react with the VOCs in an air or breath sample introduced into a flow tube. Comparing the mass of the product ions in a database, the identity of the sample VOCs can be established. Changes in VOC concentration over time can provide an indication of disease progress. In this study, ammonia is the VOC of interest, since blood and breath ammonia increase in impaired renal function.

2. EXPERIMENTAL METHOD

Female Sprague-Dawley rats (280-320g, n=8) were housed at 21°C in a 12 hour light/dark cycle, and allowed free access to food and water. Under ketamine/domitor anaesthesia, an in-dwelling cannula was inserted into the jugular vein for the purposes of fast serial blood sampling. GFR was monitored via plasma creatinine, SIFT-MS breath sampling, and bolus inulin clearance for 5 days while the animal recovered from surgery. In a subsequent surgery, the renal arteries were exposed via a single mid-ventral incision, and clamped for 60 minutes, creating ARF via an ischemic event. GFR was monitored for 7 days using the same 3 techniques, as the animal recovered and renal function returned to normal levels. The GFR monitoring schedule is shown in Table 1.

Table 1. GFR Monitoring Schedule

Method	Monitoring Time Points	
	Post-Cannulation	Post-ARF
Plasma Creatinine	6hr, 2day	1hr, 6hr, 20hr, 30hr, 2day, 4day, 7day
Inulin Clearance	6hr, 2day	6hr, 30hr, 2day, 4day, 7day
SIFT-MS Breath	-4hr, 1hr, 6hr, 20hr, 30hr, 2 day, 5 day	-4hr,1hr, 6hr, 20hr, 30hr, 2day, 3day, 4day,5day, 7day

2.1 Radio-labelled Inulin Clearance

$_{14}$C labelled inulin (1 µCi of 1.12 µCi/mg dissolved in 0.1mL ionised water, diluted 1:4 with saline) was injected into the jugular vein cannula of a conscious animal over 2 seconds, and flushed with heparinised saline. A 150µL blood sample was collected via the jugular vein cannula at -1, 1.5, 3, 6, 12, 20, 40, 70 minutes post-bolus, and sent for scintillation analysis. At 1 minute prior to injecting the bolus, an additional 150µL blood was collected for creatinine analysis. Red cells and remaining blood plasma were returned to the animal via the jugular vein after each experiment.

2.2 Breath Sampling via SIFT-MS

Direct breath was collected from conscious rats by wrapping the rat in a towel and placing it into a 300mL bottle (Fig. 2).

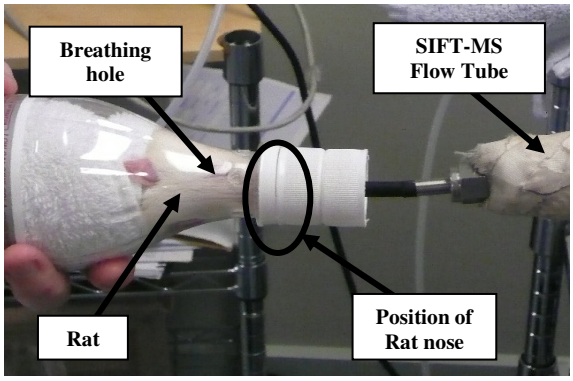

Fig. 2. Breath Collection Apparatus

A background air sample was also obtained at each rat breath sample time-point. The tightness of the fit in the bottle combined with wrapping the rat in a towel minimized contamination from the fur and waste products. Breathing holes were located posterior to the position of the rats' nose. Breath was drawn directly from the bottle through the SIFT-MS machine at 2mL/sec via a small straw attached to the end of the bottle. Because rats of this size have a breath flow rate of 2-3mL/sec, background air plays a significant role.

3. MATHEMATICAL MODELS

3.1 Inulin Clearance

Injection of an inulin bolus into the blood is described by the 2-compartment model in Fig. 3, where the bolus is injected into the plasma compartment, *P*, (with distribution volume V_P), and moves bi-directionally to the interstitial compartment, *Q*, (distribution volume V_Q), before being eliminated from the plasma via the kidneys. It is assumed that the bolus is given so quickly that the concentration in the interstitial compartment is initially zero. This assumption also indicates that the initial concentration in the plasma compartment is determined from the size of the injected bolus and the plasma volume. Hence, *u(t)* is set to zero.

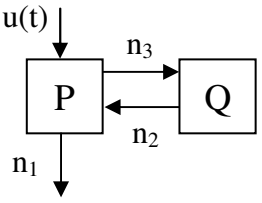

Fig. 3. Two-Compartment Model of Kidney Function

The differential equations describing inulin in the 2 compartments are dependent on the rate constants n_1, n_2, and n_3, and the inulin mass in both compartments, *p(t)* and *q(t)*.

$$\frac{d}{dt}p(t) = -(n_1 + n_3)\,p(t) + n_2 q(t) \tag{2}$$

$$\frac{d}{dt}q(t) = -n_2 q(t) + n_3 p(t) \tag{3}$$

In terms of concentrations, where $P(t) = \frac{p(t)}{V_P}$ and $Q(t) = \frac{q(t)}{V_Q}$, and defining $\alpha = \frac{V_Q}{V_P}$, (2) and (3) become:

$$\dot{P}(t) = -n_1 P(t) - n_2 \alpha (P(t) - Q(t)) \tag{4}$$

$$\dot{Q}(t) = n_2 (P(t) - Q(t)) \tag{5}$$

Parameters n_1 and n_2 were identified using an iterative integral fitting method, (Hann et al., 2005). The assumption that the concentration in the interstitial compartment is initially zero is verified by the -1 minute blood sample. First, (4) is integrated to obtain an approximation to *P(t)* in terms of the unknowns n_1 and n_2. A system of equations is then set up (6), and solved for n_1 and n_2 using linear least squares.

$$\left[\int P(t)dt \quad \alpha\int (P(t) - Q(t))dt\right]\begin{bmatrix} n_1 \\ n_2 \end{bmatrix} = \left[P(0) - P(t)\right] \tag{6}$$

The initial approximation of *P(t)* is a linear piecewise approximation obtained from the measured time and concentration data, and *P(0)* is the estimated average inulin concentration in the plasma immediately after injection of the bolus, defined as the product of the bolus volume and concentration, divided by the estimated plasma volume of the animal based on its weight. Using the obtained solution for n_1 and n_2, a new approximation for *P(t)* is generated. Constraints are added to the least squares solution to keep the approximation within known physiological ranges. Given the approximation for *P(t)*, *Q(t)* can be solved for analytically using the convolution integral (7).

$$Q(t) = n_2 e^{-n_2 t}\int P(\tau) e^{n_2(t-\tau)} d\tau \tag{7}$$

Iterations continue until convergence is achieved. The method is repeated for α values within the physiologically reported range of 2.2 – 4 (Levitt, 2003), until a least squares solution is found. The GFR is then equal to the product of n_1 and the plasma volume, where plasma volume is defined:

$$V_P = \frac{-bolus \times raw\_cts \times (-1 + e^{-n_1 t\_bolus})}{n_1 \times P_0 \times t\_bolus \times e^{-n_1 t\_bolus}} \tag{8}$$

where *bolus* is the volume of inulin solution injected, *raw_cts* is the concentration of the injected inulin, *t_bolus* is the time over which the bolus was injected, and *P(0)* is the average inulin concentration in the plasma immediately after injecting the inulin bolus.

3.2 Plasma Creatinine

The Cockcroft-Gault equation for GRF in humans is:

$$GFR = \frac{(140 - age)(wt)(0.85 \;\; if \;\; female)}{72\,Cr} \tag{9}$$

where *age* is patients age (years), *wt* is their weight (kg), and *Cr* is the plasma creatinine (μmol/L). All animals in the present study are female and approximately the same age, therefore by plotting GFR *(mL/min)* values generated using the inulin clearance method against $\frac{wt\,(kg)}{Cr\,(\mu mol\,/\,L)}$ for all rats at all time points, Fig. 4 and (10) are obtained, where the Pearson product-moment correlation coefficient (R-value) is 0.94.

$$GFR = 220\,\frac{wt}{Cr} \tag{10}$$

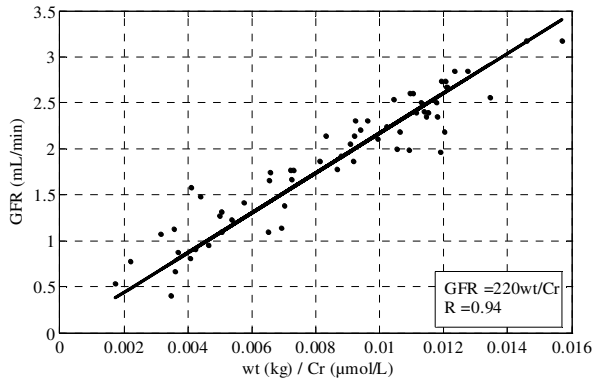

Fig. 4. Relationship between Creatinine, Weight and GFR

3.3 SIFT-MS Breath Sampling

A pilot study was conducted to determine the relationship between rat breath and background air in the experimental set-up described in Section 2.2. Healthy, conscious rats were allowed to equilibrate in a room containing a controlled concentration of ammonia. By increasing the concentration of the background ammonia, C_B, and measuring the sample collected in the bottle, C_{Tot}, Fig. 5 was obtained.

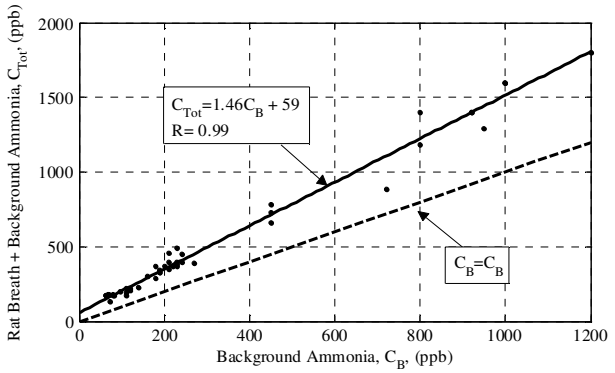

Fig. 5. Relationship between Background and Breath in healthy rats

Linear regression yields (11), where the x-intercept represents the concentration of the analyte in breath given a zero concentration in background air. The slope of C_{Tot} greater than 1 indicates that ammonia is concentrated in the breath against its concentration gradient from the blood, a finding that has not been previously described.

$$C_{Tot} = 1.46 \times C_B + 59 \tag{11}$$

A lumped parameter model for the breath circuit is shown in Fig. 6. In this model, the rat is breathing at a volume flow rate of V_R ml/sec, and breathes in and out concentrations of C_{Tot} and C_R, respectively. SIFT-MS draws the sample at 2mL/sec, and thus background air must be drawn into the bottle at 2mL/sec to maintain equilibrium. The volume flow rate of rat breath is calculated from tidal volume and respiratory rate, which for a 250-300g rat, is approximately 2.7mL/sec (Pass and Freeth, 1993).

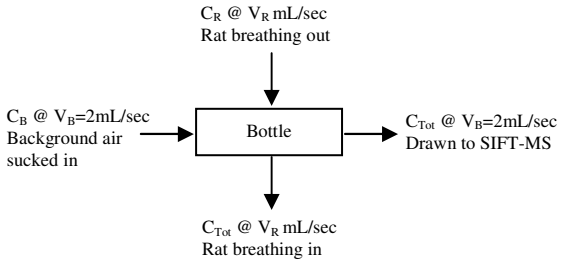

Fig. 6. Rat Breathing Circuit

Using continuity of mass, C_R can be calculated from (12).

$$\begin{aligned} & C_B V_B + C_R V_R = C_{Tot} V_B + C_{Tot} V_R \\ \Rightarrow\ & C_R = (1 + \frac{V_B}{V_R}) C_{Tot} - (\frac{V_B}{V_R}) C_B \end{aligned} \tag{12}$$

Plotting C_R against C_B and fitting a linear regression as in Fig. 5 and (11), yields:

$$C_R = 1.79 \times C_B + 102 \tag{13}$$

Note that the C_R obtained from (12) is specific to the background air, C_B, and must be normalized to a zero background air concentration using (13):

$$C_{R^*} = \frac{(1 + \frac{V_B}{V_R}) C_{Tot} - (\frac{V_B}{V_R}) C_B}{102 + 1.79 C_B} \tag{14}$$

C_{R^*} was plotted against time, following the course of each surgical intervention. Hence, each rat provided their own control from the first surgery to ensure that it was the ARF and not surgery alone causing the rise in breath ammonia. Using this method, a percentage increase in breath ammonia can be reported for each rat. Alternatively, breath ammonia can be converted to GFR by plotting GFR obtained via inulin clearance against C_{R^*} for all rats at all time-points (Fig. 6). Hence, GFR can be estimated using breath ammonia using (15). Note that domitor causes a 70% depression in respiration. Therefore, the ratio V_B/V_R increases during surgery and recovery from anesthesia.

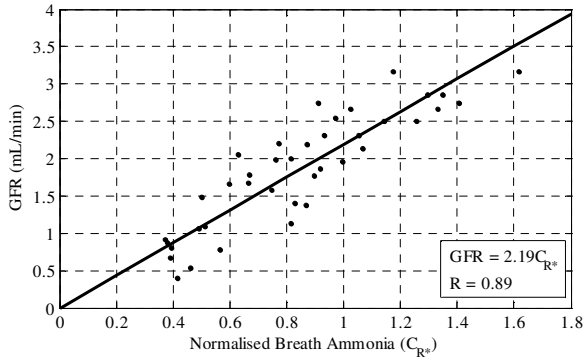

Fig. 6. Relationship between Breath Ammonia and GFR

$$GFR = \frac{2.19}{C_{R^*}} \quad (15)$$

4. RESULTS AND DISCUSSION

4.1 Bolus Radio-labelled Inulin Clearance Test

Using the iterative integral fitting method, clearance curves were obtained for each rat at each time-point. Due to the difficulty in keeping the jugular vein cannula patent for 12 days, some animals did not undergo the full contingent of 7 inulin clearance tests. Fig. 7 shows a typical inulin clearance curve obtained for a rat in the normal and ARF states. The average curve fitting error was less than 10%.

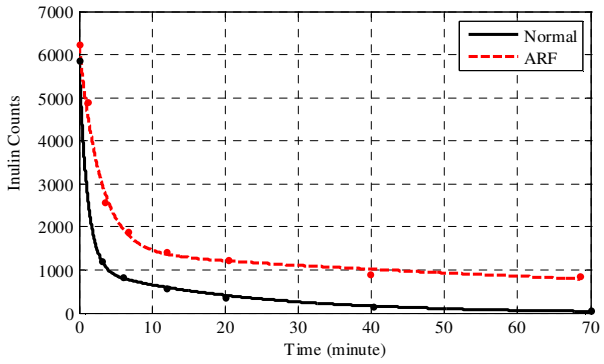

Fig. 7 Inulin Clearance Curve

Table 2 summarizes the inulin clearance test results. The maximum decrease in GFR was observed in the first clearance test post-ARF, at the 6 hour time-point.

Table 2. Inulin GFR Estimation

Rat	Weight (g)	Estimated GFR		% decrease	Model % error
		Normal	ARF		
1	299	2.60	0.95	63	8.2
2	300	2.50	0.40	84	7.3
3	300	2.67	0.81	70	9.1
4	302	2.39	1.1	54	9.1
5	291	2.74	0.91	67	8.9
6	300	2.55	-	-	12.7
7	306	3.17	1.09	66	8.4
8	312	2.86	0.68	76	7.3

4.2 Plasma Creatinine

Maximum changes in plasma creatinine concentrations were found to occur between 6 and 20 hours post induction of ARF, as indicated in Table 3. Estimated GFR values obtained from (10) are shown in Table 3, where the fitting factor refers to the constant in (10) when data is fitted in a rat-specific manner, as opposed to across the tested rat population. This deviation in fitting factor gives some indication of the expected error in the GFR estimation when compared to the inulin-clearance estimated GFR.

Table 3. Creatinine GFR Estimation

Rat	Estimated GFR		% decrease	Fitting factor	Hours to max. conc.
	Normal	ARF			
1	2.59	1.09	58	235	6
2	2.56	0.33	87	217	20
3	2.69	0.90	67	222	6
4	2.49	1.21	51	243	20
5	2.63	0.92	65	210	6
6	2.33	0.88	62	240	30
7	3.25	1.05	68	207	6
8	2.78	0.78	72	212	6

4.3 SIFT-MS Breath Analysis

Selected Ion Mode (SIM) scans for ammonia were performed on rat breath samples, and converted to GFR estimates as summarized in Table 4. The decreases (%) in renal function results show excellent correlation with the inulin estimations of Table 2, with a Pearson product-moment correlation coefficient of 0.89.

Table 4. Breath Ammonia GFR Estimation

Rat	Estimated GFR		% decrease	Hours to max. conc.
	Normal	ARF		
1	2.56	1.09	57	6
2	2.75	0.42	85	20
3	2.92	0.86	71	6
4	2.35	1.17	50	6
5	3.08	0.82	73	6
6	2.94	0.71	76	6
7	3.13	1.44	54	6
8	2.96	0.85	71	6

Error in the GFR estimation is due to the oscillation in ammonia concentration as recorded by the SIFT-MS, and seen in Fig. 8. A probably density function can be formed around the concentration data, to obtain the inter quartile range of ammonia concentration. Because of the similar flowrates between breath and machine sampling, the error is effectively amplified in the current study when C_{Tot} and C_B are combined to obtain C_R. However, with the introduction of a new *Voice*200® SIFT-MS machine which samples at the lower rate of 2mL/min (as opposed to 2mL/sec), this error could be minimized in future studies.

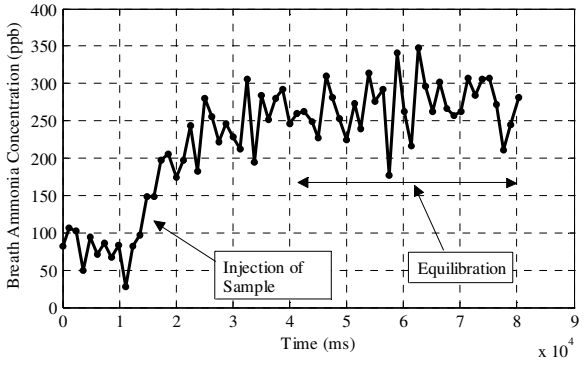

Fig. 8. Oscillation in Ammonia SIM Scan

4.4 Comparison in GFR Estimations

Excellent correlation is observed between the percentage decreases in renal function estimated by the 3 different methods. When the population metrics are combined for the plasma creatinine and breath ammonia data to obtain GFR estimates, Figs. 9-11 are obtained for a selection of the animals.

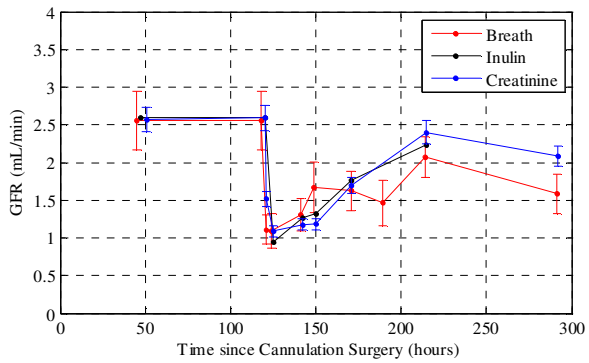

Fig. 9. Rat 1 GFR Comparision

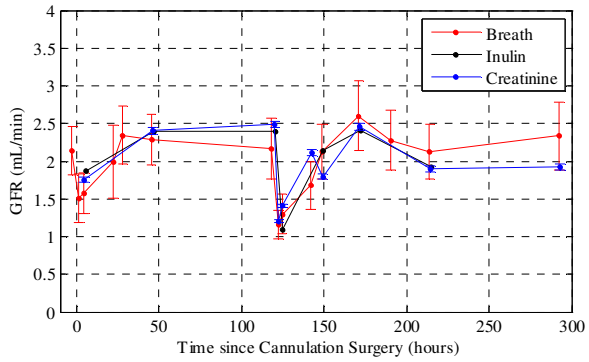

Fig. 10. Rat 4 GFR Comparision

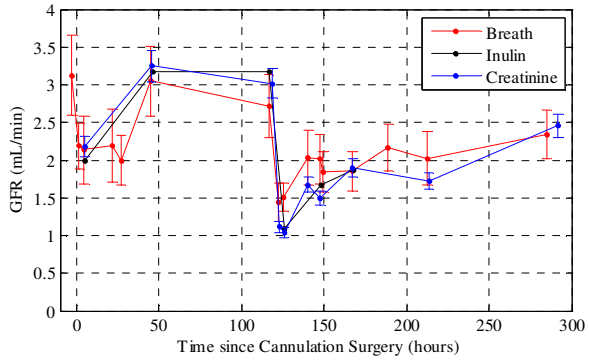

Fig. 11. Rat 7 GFR Comparision

Figures 9-11 show the time-course for GFR estimation via the 3 methods. An initial decrease in GFR is caused by surgery alone, and the second, larger decrease in GFR is due to the ARF. Error bars indicate the variability of SIFT-MS. Better technology with the *Voice*200®, and filtering of multiple samples would reduce this error and further improve correlations.

5. CONCLUSIONS

ARF was induced in 8 rats, and the degree of renal failure was estimated using 3 different methods: bolus radio-labelled inulin clearance test, plasma creatinine measurement, and SIFT-MS breath analyte monitoring. The relative decrease in function is the most useful metric, for which the Pearson product-moment correlation coefficient was 0.89 between breath and inulin techniques. However, a population model for absolute GFR estimation was also able to be generated from creatinine and breath data with very good correlations observed in Figures 9-11.

It was found in a pilot study (Figure 5), that the concentration of expired ammonia increases as a function of background ammonia concentration with a slope greater than 1. This result indicates that ammonia can be removed from the body at a concentration greater than that possible by simple diffusion from the blood, alone, suggesting the presence of ammonia transporters in lung epithelium for actively transporting ammonia.

Determination of renal function via SIFT-MS is desirable since results are available non-invasively and in real time. Correlation between relative decreases in renal function presented here, indicate good promise for fast, non-invasive determination of renal function via breath testing.

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