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**Factors that confound the prediction of renal medullary oxygenation and risk of acute kidney injury from measurement of bladder urine oxygen tension**

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**Running title:** Urinary and medullary oxygen tension

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**ABSTRACT**

**Aim:** Urinary oxygen tension ( $uPO_2$ ) may provide an estimate of renal medullary  $PO_2$  ( $mPO_2$ ) and thus risk of acute kidney injury (AKI). We assessed the potential for variations in urine flow and arterial  $PO_2$  ( $aPO_2$ ) to confound these estimates.

**Methods:** In 28 sheep urine flow,  $uPO_2$ ,  $aPO_2$ , and  $mPO_2$  were measured during development of septic AKI. In 65 human patients undergoing cardiac surgery requiring cardiopulmonary bypass (CPB)  $uPO_2$  and  $aPO_2$  were measured continuously during CPB, and in a subset of 20 patients, urine flow was estimated every 5 min.

**Results:** In conscious sheep breathing room air,  $uPO_2$  was more closely correlated with  $mPO_2$  than with  $aPO_2$  or urine flow. The difference between  $mPO_2$  and  $uPO_2$  varied little with urine flow or  $aPO_2$ . In patients, urine flow increased abruptly from  $3.42 \pm 0.29 \text{ mL min}^{-1}$  to  $6.94 \pm 0.26 \text{ mL min}^{-1}$  upon commencement of CPB, usually coincident with reduced  $uPO_2$ . During hyperoxic CPB high values of  $uPO_2$  were often observed at low urine flow. Low urinary  $PO_2$  during CPB ( $<10 \text{ mmHg}$  at any time during CPB) was associated with greater (4.5-fold) risk of AKI. However, low urine flow during CPB was not significantly associated with risk of AKI.

**Conclusions:**  $uPO_2$  provides a robust estimate of  $mPO_2$ , but this relationship is confounded by the simultaneous presence of systemic hyperoxia and low urine flow. Urine flow increases and  $uPO_2$  decreases during CPB. Thus, CPB is probably the best time to use  $uPO_2$  to detect renal medullary hypoxia and risk of post-operative AKI.

**Keywords:** Acute kidney injury, cardiac surgery, cardiopulmonary bypass, hypoxia, sepsis

## INTRODUCTION

It has been proposed that hypoxia in kidney tissue, particularly in the renal medulla, contributes to the development of acute kidney injury (AKI),<sup>1</sup> including cardiac surgery associated AKI.<sup>2</sup> It is not feasible to routinely or directly measure renal medullary tissue oxygen tension ( $PO_2$ ) in humans. However, it is possible to measure the  $PO_2$  of bladder urine in patients with an indwelling bladder catheter.<sup>3, 4</sup> In a pioneering study, Kainuma et al continuously measured bladder urinary  $PO_2$  ( $uPO_2$ ), in patients undergoing cardiac surgery requiring cardiopulmonary bypass (CPB), using polarographic electrodes.<sup>3</sup> They observed a reduction in  $uPO_2$  during CPB. Furthermore, both the rate and amplitude of recovery of  $uPO_2$  after CPB were inversely correlated with peak post-operative serum creatinine concentration (i.e. renal dysfunction). More recently, Zhu et al, using fiber optic probes, confirmed that  $uPO_2$  falls during cardiac surgery requiring CPB.<sup>4</sup> Furthermore, they found that low  $uPO_2$  during the surgical procedure predicted subsequent development of post-operative AKI.<sup>4</sup> We propose that the findings of both Kainuma et al<sup>3</sup> and Zhu et al<sup>4</sup> reflect a close association between renal medullary  $PO_2$  ( $mPO_2$ ) and  $uPO_2$ . This proposition is supported by recent experimental observations, in both sheep<sup>5-8</sup> and rabbits,<sup>9</sup> indicating that changes in renal medullary  $PO_2$  ( $mPO_2$ ) can be detected from changes in  $uPO_2$ . Nevertheless, diffusion of oxygen between the urine and the urothelium of the ureter and bladder confounds the relationship between renal  $mPO_2$  and  $uPO_2$ .<sup>9</sup> Consequently, our ability to infer changes in  $mPO_2$  from changes in  $uPO_2$  could be affected by both urine flow and arterial  $PO_2$  ( $aPO_2$ ).

In the current study we exploited data we had previously generated, from experimental studies in ovine septic AKI<sup>5-7</sup> and in patients undergoing cardiac surgery requiring CPB,<sup>4</sup> to perform a detailed analysis of the influences of urine flow and arterial  $PO_2$  ( $aPO_2$ ) on  $uPO_2$ . In sheep, intravenous infusion of *Escherichia coli* consistently causes sepsis and AKI.<sup>5-7</sup> Reductions in both  $mPO_2$  and  $uPO_2$  are early and persistent events in this model, preceding increased serum creatinine and reduced urine flow by many hours.<sup>6</sup> Thus, re-analysis of the data we had collected previously<sup>5-7</sup> provided an opportunity for us to assess the potentially confounding influences of variations in urine flow and  $aPO_2$  on our ability to infer  $mPO_2$  from measurement of  $uPO_2$ . Our observations in patients undergoing cardiac surgery requiring CPB included continuous measurement of  $uPO_2$  and  $aPO_2$  while the patients were on CPB. Additionally, for the purposes of the current study, we developed a method for virtually continuous measurement of urine flow in the operating theatre. This then allowed us

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to investigate the potential for simultaneous measurement of urine flow and  $uPO_2$ , during CPB, to improve prediction of AKI over that provided by measurement of each variable on its own.

## RESULTS

### Experimental studies in ovine septic acute kidney injury

The data presented in this study were generated from measurements of  $mPO_2$ ,  $uPO_2$ ,  $aPO_2$ , and urine flow from three previously published studies of renal and urinary oxygenation in a total of 28 adult Merino ewes.<sup>5-7</sup> In the current study we assessed the relationships between  $mPO_2$ ,  $uPO_2$ ,  $aPO_2$ , and urine flow in these sheep. Detailed descriptions of the methods are provided in these previous reports and are described briefly in the Materials and Methods section. This data-set provides observations of simultaneously measured  $uPO_2$  and  $mPO_2$  under a range of pathophysiological conditions. Detailed findings have previously been reported<sup>5-7</sup> and are briefly summarized below.

Simultaneously measured  $uPO_2$  and  $mPO_2$  fell markedly within the first hour of infusion of *Escherichia coli*. Sepsis was also associated with an initial diuresis (from a baseline of  $\sim 1$  mL  $\text{min}^{-1}$  to a maximum of  $\sim 2$  mL  $\text{min}^{-1}$ ) during the first  $\sim 6$  h of infusion of *Escherichia coli* followed by relative oliguria ( $<0.5$  mL  $\text{min}^{-1}$ ). Arterial  $PO_2$  fell from  $\sim 90$  mmHg to  $\sim 80$  mmHg. The effects of the various treatments on these variables are described in the original articles.<sup>5-7</sup> The number of data points for each variable varied across the four groups of sheep. For the sheep treated with saline or norepinephrine,  $mPO_2$ ,  $uPO_2$ , and urine flow were recorded for 41 one-hour time-periods in each sheep.<sup>5</sup> Arterial  $PO_2$  was recorded at 8 time-points.<sup>5</sup> For sheep treated with angiotensin II or saline vehicle,  $mPO_2$  and  $uPO_2$  were recorded over 8 one-hour time-periods while urine flow and  $aPO_2$  were recorded during 5 time periods.<sup>6</sup> Lastly, for the volume resuscitation protocol  $mPO_2$  and  $uPO_2$  were recorded over 14 one-hour time-periods while urine flow and  $aPO_2$  were recorded during 11 time-periods.<sup>7</sup>

In the current analysis, we detected a positive correlation between  $mPO_2$  and  $uPO_2$  (Fig. 1A). The 95% confidence interval (CI) of the slope (0.96 to 1.11) included unity and the 95% CI of the  $y$ -intercept (-0.08 to 3.78) included zero. The calculated linear relationship accounted for 29% of the variation in  $uPO_2$ . The mean level of  $uPO_2$  varied in the different sheep ( $P_{\text{sheep}} < 0.001$ ), as did the slope of the relationship between  $uPO_2$  and  $mPO_2$  ( $P_{\text{sheep} * \text{medullary } PO_2} < 0.001$ ). When these factors were included in the model (full regression model), it accounted

for 56% of the variation in  $uPO_2$ . There were weak but statistically significant linear relationships between urine flow and  $uPO_2$  (Fig. 1B) and  $aPO_2$  and  $uPO_2$  (Fig. 1C). However, the calculated linear relationships accounted for  $\leq 6\%$  of the variation in  $uPO_2$ .

Across all time-points, the mean between-sheep difference between simultaneously measured  $mPO_2$  and  $uPO_2$  was  $-1.3 \pm 0.2$  mmHg ( $n=28$ ). There was a weak but statistically significant linear relationship between urine flow and the difference between  $mPO_2$  and  $uPO_2$  but this accounted for only 1% of the variance in the dataset (Fig. 2A). There was no significant relationship between  $aPO_2$  and the difference between  $mPO_2$  and  $uPO_2$  (Fig. 2B).

### **Observational studies in patients undergoing cardiac surgery on cardiopulmonary bypass**

*Optimization of gravimetrically determined urine flow:* In our previously published report on 65 patients undergoing cardiac surgery requiring CPB,<sup>4</sup> a bladder catheter was inserted and  $uPO_2$  was measured via a fiber optic probe. The patient's urine bag was placed on an electronic scale underneath the patient's head. The scale was connected wirelessly to a computer so that the weight of the urine bag was recorded every 5 seconds. These gravimetric measurements of urine flow were not included in the previously published report.<sup>4</sup> Systemic  $aPO_2$  was also measured continuously while patients were on CPB. For every patient, the surgical procedure was divided into four 'epochs': (i) 'pre-bypass': the period between the first incision and commencing CPB, (ii) 'hypothermic CPB': during CPB when patients were cooled to 33-34 °C, (iii) 'rewarming': when patients were rewarmed to 36-37 °C before weaning from CPB, and (iv) 'post-bypass': the period from coming off CPB until the end of the surgical procedure.

Volumetric urine flow was determined by dividing the volume of urine by the duration of the epoch.

Gravimetric urine flow was determined by calculating the change in the weight of the urine bag every 5 seconds. We termed this 'raw' urine flow. Because the weight of the urine bag could change independently of urine flow, we evaluated two different approaches to cleaning the data, herein termed 'disturbance-based', and 'inclusion criteria-based'. For the disturbance-based approach, known disturbances to the urine bag or electronic scale, as well as the time at which they occurred, were recorded in the operating theatre. The weights corresponding to the disturbances were then removed prior to calculations of urine flow. The

mean urine flow for each epoch was then calculated once all these disturbances (see Materials and Methods) were accounted for. For the inclusion criteria-based approach, values of urine flow that fell outside specific ranges were excluded from the data-set.

In a subset of 20 of the 65 patients included in our previous report,<sup>4</sup> we conducted an additional analysis involving a comparison between the volumetric and gravimetric methods for determining urine flow. For each of the four epochs, mean urine flow determined gravimetrically was plotted against urine flow determined volumetrically. Thus, in the current analysis a total of 80 sets of observations were generated from the subset of 20 patients. Ordinary least products (OLP) regression was used to generate lines of best fit.

Regression analysis of the raw data failed to generate a significant linear relationship between volumetrically and gravimetrically determined urine flow (Table 1). When the data were cleaned using the disturbance-based approach, the line of best fit only accounted for 17% of the variation in gravimetric urine flow.

A range of inclusion criteria were applied to the raw 5 s data (Table 1, Fig. 3). Empirically, the best criterion was  $-10$  to  $50 \text{ mL min}^{-1}$ , which accounted for 78% of the variation in gravimetric urine flow. Furthermore, 86% of the original raw data were retained. This method was then employed in all subsequent analyses.

*Temporal resolution of gravimetrically determined urine flow:* Estimates of urine flow at 5 s intervals, even after removal of values outside the inclusion criteria ( $-10$  to  $50 \text{ mL min}^{-1}$ ), were highly variable (Fig. 4). Variability was considerably less over 1 min periods, but estimates of urine flow were still occasionally physiologically implausible. In contrast, estimates of urine flow determined at 5 min intervals were consistently within a physiological range. Thus we reasoned that our method allows urine flow to be resolved to 5-minute periods.

*Temporal variations in  $u\text{PO}_2$ , urine flow and  $a\text{PO}_2$  during cardiac surgery:* Urine flow increased during CPB (Fig. 5). In some patients, it increased abruptly upon commencement of CPB while in others it increased more slowly. Urine flow in most patients returned to close to pre-bypass levels after weaning from CPB. Arterial  $\text{PO}_2$  was high and generally stable during CPB (Fig. 5), reflecting the use of 60% oxygen as the gas feed to the oxygenator in the perfusion circuit.

Urinary PO<sub>2</sub> commonly fell abruptly at the onset of CPB. In some patients the fall in uPO<sub>2</sub> preceded the increased urine flow (Figs 5B, D & E), in some patients the two phenomena occurred simultaneously (Fig. 5A) and in some urine flow increased before uPO<sub>2</sub> fell (Fig. 5C & F).

During CPB there was a weak but statistically significant linear relationship between urine flow and uPO<sub>2</sub> (Fig. 6A), accounting for 18% of the variance in uPO<sub>2</sub>. The relationship appeared exponential, with very high levels of uPO<sub>2</sub> often observed at lower rates of urine flow (e.g. <3 mL min<sup>-1</sup>). No significant linear relationship could be detected between aPO<sub>2</sub> and uPO<sub>2</sub> (Fig. 6B).

*Urine flow and uPO<sub>2</sub> during CPB and the risk of post-operative AKI:* We re-analysed data from our previous report,<sup>4</sup> of 65 patients undergoing cardiac surgery requiring CPB, to assess various thresholds of urine flow (measured volumetrically) and uPO<sub>2</sub> during CPB as predictors of post-operative AKI (Table 2). This analysis differed from that in our previous report,<sup>4</sup> in which uPO<sub>2</sub> and urine flow were considered across the entire course of the surgical procedure. None of the thresholds of urine flow during CPB we assessed, ranging from <3.0 mL min<sup>-1</sup> to <1 mL min<sup>-1</sup>, had significant predictive efficacy. In contrast, risk of post-operative AKI was increased in patients whose uPO<sub>2</sub> fell to <10 mmHg (4.54-fold), < 7.5 mmHg (6.42-fold) or <5 mmHg (6.82-fold). Risk of AKI was also greater in patients who experienced either urine flow <2 mL min<sup>-1</sup> or uPO<sub>2</sub> <10 mmHg (5.73-fold), but not significantly greater than that in patients who only fulfilled the uPO<sub>2</sub> criteria.

## Discussion

We used data-sets from previously published experimental and clinical studies to explore the potential for estimates of mPO<sub>2</sub>, generated from measurement of bladder uPO<sub>2</sub>, to be confounded by differences in urine flow and aPO<sub>2</sub>. In conscious sheep, breathing room air, uPO<sub>2</sub> varied linearly with mPO<sub>2</sub> but was only weakly associated with urine flow and aPO<sub>2</sub>. Variations in urine flow or aPO<sub>2</sub> did not systematically or appreciably confound estimation of mPO<sub>2</sub> from measurement of uPO<sub>2</sub> under these experimental conditions. Nevertheless, in humans during hyperoxic CPB, the variability of uPO<sub>2</sub> was greater at lesser urine flow. Thus, the ‘information value’ of uPO<sub>2</sub> depends on the level of urine flow, particularly under the hyperoxic conditions often employed during major surgery. Consequently, we developed a new method that allows urine flow to be temporally resolved to 5-minute intervals in the human operating theatre. We found that urine flow commonly increases and uPO<sub>2</sub> falls upon

commencement of CPB. In a sample of 65 patients undergoing cardiac surgery, low urinary  $PO_2$  during CPB, but not low urine flow during CPB, was significantly associated with development of post-operative AKI. Thus, our current findings provide further support for the utility of  $uPO_2$  as a real-time intra-operative marker of risk of cardiac-surgery associated AKI. However, addition of simultaneous continuous measurement of urine flow may have limited benefit.

Predictions from a mathematical model of oxygen diffusion across the urothelium indicate that urine flow and systemic oxygenation are the major confounders of the relationship between  $uPO_2$  and  $mPO_2$ .<sup>9</sup> At lesser urine flow there is a longer ureteric transit-time and thus greater time available for diffusion of oxygen between urine and the ureteric wall. Furthermore, the driving force for diffusion of oxygen between these two compartments is the  $PO_2$  difference between them. Our current study was directed towards understanding how much these confounders actually matter and to generate strategies to overcome them.

Our current analysis confirms the good agreement between  $uPO_2$  and  $mPO_2$  in sheep during development of septic AKI and resuscitation with norepinephrine,<sup>5</sup> angiotensin II<sup>6</sup> or crystalloid.<sup>7</sup> It also indicates that, at least under the normoxic or slightly hypoxic conditions in which the sheep were studied, variations in urine flow and  $aPO_2$  have relatively little impact on the accuracy of estimates of  $mPO_2$  generated by measurement of  $uPO_2$ . In contrast,  $uPO_2$  does appear to be influenced by urine flow in patients undergoing cardiac surgery. Our observations are of course limited by the fact that we could not measure  $mPO_2$  in humans. Furthermore, our observations were made in patients under conditions of hyperoxemia, as is standard in human cardiac surgery.<sup>4</sup> Indeed, the fact that variations in  $uPO_2$  were skewed towards higher levels of  $uPO_2$  at lower urine flow probably reflects the hyperoxemic status of the patients. That is, longer ureteric transit time provides greater time for diffusion of oxygen from the hyperoxic ureteric wall into the urine, increasing the  $PO_2$  of urine in the bladder.

Due to the potential for urine flow to confound estimation of  $mPO_2$  from  $uPO_2$  during major surgery, we developed a new method for measurement of urine flow in patients in the operating theatre which provides relatively high temporal resolution. Multiple monitoring devices for continuous measurement of urine output have been developed.<sup>10-13</sup> For example, Chang et al recently described a similar approach to ours, deployed in 30 patients undergoing cardiac surgery, but provided no analysis of the temporal resolution of the method.<sup>14</sup> Because of the large dead space in clinically used urine collection systems there is potential for urine

to collect in the tubing and so enter the urine collection bag periodically. Indeed, based on the large fluctuations in urine weight observed in the data we collected every 5 s, this appears to be the case. Thus, it is imperative that these data are averaged over time periods that allow these fluctuations to be averaged out. Based on our current analysis we are confident that our method provides relatively accurate measurements of urine flow over 5 min periods. Thus, it is feasible to generate real-time measurement of  $uPO_2$  and near real-time measurement of urine flow during cardiac or other major surgery.

Urine flow increased during CPB in human patients. The resultant decrease in ureteric transit time might be expected to mitigate diffusion of oxygen from the ureter to urine, thus leading to reduced  $uPO_2$ . Indeed,  $uPO_2$  did fall during CPB. However, the fall in  $uPO_2$  sometimes preceded increased urine flow. Thus, while some of the reduction in  $uPO_2$  observed during CPB may result from increased urine flow, it seems likely that it also reflects a signal of reduced renal  $mPO_2$ . This conclusion is consistent with available observations of CPB in experimental animals, which is associated with renal medullary hypoxia.<sup>15-17</sup> It is also consistent with the observation of increased circulating levels of erythropoietin in human patients soon after CPB.<sup>18</sup>

‘Minute-by-minute’ measurement of urine output has been proposed for earlier detection of AKI.<sup>19</sup> In the context of cardiac surgery, we recently found that risk of post-operative AKI is independently associated with both a lesser intra-operative urine flow and a lesser intra-operative  $uPO_2$ .<sup>4</sup> Yet our current analysis shows that  $uPO_2$  falls and urine flow increases on bypass. Therefore, the simultaneous measurement of urine flow and  $uPO_2$  during CPB, the period of cardiac surgery arguably most amenable to intervention to ameliorate risk of AKI, might be expected to provide better assessment of the risk of post-operative AKI than either urine flow or  $uPO_2$  alone. However, using a range of thresholds of urine flow during CPB, we were unable to detect significantly increased risk of AKI associated with low urine flow on CPB. In contrast, low  $uPO_2$  during CPB was associated with significantly increased risk of AKI. We must acknowledge that these observations were made in a relatively small sample of 65 patients for whom we had measurement of urine flow and  $uPO_2$  during CPB.<sup>4</sup> Thus, while we cannot exclude the possibility that urine flow during CPB is associated with post-operative AKI, our current observations provide no support for this hypothesis.

We acknowledge a major limitation of our current analysis. In our studies in sheep we have been unable to identify specific ranges of urine flow or  $aPO_2$ , beyond which  $uPO_2$  no longer

provides a reliable estimate of  $mPO_2$ , or in the setting of cardiac surgery, risk of AKI. We think the challenge of predicting  $mPO_2$  from  $uPO_2$  will best be met by development of a sophisticated computational model of oxygen transport across the ureteric wall. Such a model could provide both a method to predict  $mPO_2$  from  $uPO_2$  (deterministic) and a means to assess the uncertainty of these predictions (probabilistic). We and our collaborators have previously developed models of oxygen transport in the ureter of rats<sup>20</sup> and rabbits.<sup>9</sup> The next step is development of models based on the sheep and human. The current analysis is a critical step in this process because it identifies the variables (urine flow and arterial  $PO_2$ ) that matter the most and some of the critical parameters that should be incorporated in such models. Regarding prediction of AKI from intra-operative measurement of  $uPO_2$ , our current dataset of 65 patients is too small to stratify by level of intra-operative urine flow. This might be possible with a larger dataset, enabling determination of a level of urine flow below which  $uPO_2$  does not provide prognostic information regarding the risk of post-operative AKI.

In conclusion, our observations indicate that  $uPO_2$  provides a relatively reliable estimate of  $mPO_2$ . However, the relationship between  $uPO_2$  and  $mPO_2$  may be confounded by low urine flow and hyperoxemia, particularly when they occur simultaneously. In patients undergoing cardiac surgery, urine flow is commonly high during CPB, making this a good time to use  $uPO_2$  to detect the presence of hypoxia in the renal medulla. Indeed, our data indicate that risk of AKI is significantly and markedly increased if  $uPO_2$  falls below 10 mmHg at any time during CPB. The period of CPB is also arguably the best time to intervene to prevent AKI, since the perfusionist has direct control over systemic hemodynamics.<sup>17</sup> Our findings also indicate that low urine flow during CPB is not strongly associated with development of post-operative AKI. Thus, while it is feasible to remotely measure urine flow during CPB with a temporal resolution of at least 5 min, this information may be of little additional benefit for prediction of AKI over and above continuous measurement of  $uPO_2$ .

## **METHODS**

### **Ethics**

Studies in experimental animals were approved by the Animal Ethics Committee of the Florey Institute under guidelines laid down by the National Health and Medical Research Council of Australia. All protocols for the human studies were approved by the Human Research Ethics Committees at Monash Health (approval number 12375B) and Monash University (CF16/1172 – 2016000631).

### **Experimental studies in ovine septic acute kidney injury**

*Experimental Protocol:* In each sheep, urinary and medullary PO<sub>2</sub> were averaged over 60 min periods. Urine produced by both kidneys was collected over these periods to allow calculation of urine flow. At the mid-point of each period, an arterial blood sample was collected for oximetry (ABL System 625; Radiometer Medical, Copenhagen, Denmark). Data were collected at specified time-points for 24 h under control conditions and then hourly for 32 h after commencing a continuous intravenous infusion of *Escherichia coli* to induce septic AKI ( $2.8 \times 10^9$  colony-forming units for 30 minutes as a bolus, followed by a continuous infusion of  $1.26 \times 10^9$  colony forming units h<sup>-1</sup> for 32 hours). In all sheep, fluid replacement (154 mM NaCl) was given at a rate of 1 mL kg<sup>-1</sup> h<sup>-1</sup> for the rest of the protocol. From the 24<sup>th</sup> to 30<sup>th</sup> hour after commencing the infusion of *Escherichia coli*, sheep received either an intravenous infusion of 154 mM NaCl as a vehicle control (12 mL h<sup>-1</sup>; n = 9), norepinephrine (0.4-0.8 µg kg<sup>-1</sup> min<sup>-1</sup>; Levophed, Abbott, Kurnell, Australia; n = 5), or angiotensin II (0.5-33 ng kg<sup>-1</sup> min<sup>-1</sup>; Auspep, Tullamarine, Victoria, Australia; n = 8), or volume resuscitation via a series of three boluses of 500 mL of compound sodium lactate (Hartmann's solution; Baxter Healthcare, Toongabbie, NSW, Australia; n = 6) given 24, 25 and 26 h after commencing the infusion of *Escherichia coli*.

We took advantage of the fact that septic AKI in sheep is associated with renal medullary hypoxia and that the resuscitation therapies we tested had diverse effects on mPO<sub>2</sub> and urine flow. In brief, sheep were surgically equipped with a fiber-optic probe in the renal medulla to permit continuous measurement of renal mPO<sub>2</sub> (CP-004-001; Oxford Optronix, Abingdon, UK), a catheter in a carotid artery for collection of arterial blood for oximetry, and a bladder catheter for collection of urine (size 12, 30 mL Euromedical, Malaysia), into which a fiber optic probe was placed for continuous measurement of uPO<sub>2</sub> (LAS-1/O/E, Oxford Optronix). Only sheep for which all measurements of mPO<sub>2</sub>, uPO<sub>2</sub>, aPO<sub>2</sub> and urine flow were available were included in the analysis.

### **Observational studies in patients undergoing cardiac surgery on cardiopulmonary bypass**

*Observational protocol:* These data were generated in a published observational study of 65 adult patients undergoing cardiac surgery with CPB (coronary artery bypass graft, valve surgery, or both).<sup>4</sup> Patients with a higher risk of operative mortality and post-operative AKI were recruited, as described in detail in the published report.<sup>4</sup> Patients were excluded if they (i) had pre-existing advanced chronic kidney disease (baseline serum creatinine >200 µmol L<sup>-1</sup>).

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<sup>1</sup>, or eGFR <30 mL min<sup>-1</sup> 1.73 m<sup>-2</sup>), (ii) required pre-operative hemodialysis, (iii) previously had kidney transplantation, (iv) had a confirmed pre-operative diagnosis of AKI as defined by the KDIGO AKI criteria,<sup>21</sup> (v) required off-pump surgery, or (vi) were in a critical pre-operative state so unable to provide informed consent. All participants provided written informed consent prior to enrolment to the study. Details of the management of patients, including anesthesia and perfusion, are provided in our published report of these studies.<sup>4</sup> AKI was defined according to modified KDIGO criteria<sup>21</sup>: a maximum increase in serum creatinine of  $\geq 0.30$  mg/dL (26.5 mmol L<sup>-1</sup>) from its pre-operative baseline level within the first 48 h after surgery, or  $\geq 50\%$  within the first 5 days after surgery. We did not utilize the urine output criterion for diagnosis of AKI as it may result in over-diagnosis.<sup>22, 23</sup>

We measured uPO<sub>2</sub> every 5 s using fiber optic probes similar to those used in sheep. Immediately after induction of anesthesia and insertion of a bladder catheter (Medtronic, Minneapolis, MN, USA), a sterilized fiber optic luminescence optode (NX-LAS-1/O/E-5 m; Oxford Optronix) was advanced through the lumen of the catheter so that the tip of the probe was at the tip of the catheter, in contact with urine in the bladder. The fiber optic probe was connected to a luminescence lifetime oximeter (Oxylite Pro, Oxford Optronix) interfaced with a laptop computer running LabChart software (Version 8, ADInstruments, Bella Vista, NSW, Australia), to provide a measurement of urinary PO<sub>2</sub> every 5 s during the entire surgical procedure. Systemic aPO<sub>2</sub> was also measured continuously, while patients were on-pump, using an in-line blood gas analyser (System M3, Spectrum Medical, Fort Mill, SC, USA).

The bladder catheter was connected to a standard urine collection system (Precision 400, Covidien, Minneapolis, USA). To prevent leakage of urine around the probe at the junction of the bladder catheter and the urine collection system, a drop of cyanoacrylate adhesive was placed on the probe at the point of exit from the bladder catheter. The junction of the bladder catheter and the urine collection system was then wrapped with sterile laboratory film (Parafilm, Pechiney Plastic Packaging, Menasha, WI, USA).

For every patient, the surgical procedure was divided into four epochs as described in the Results section.

*Volumetric measurement of urine flow:* Urine produced during each epoch was emptied from the patient's urine catheter bag into a measuring cylinder. Urine flow ( $\text{mL min}^{-1}$ ) was calculated by dividing the urine volume by the duration of the epoch.

*Gravimetric measurement of urine flow:* The bag, into which the patient's urine drained, was placed on an electronic balance (Mettler Toledo AG, Greifensee, Switzerland). The balance was placed, underneath the operating table, directly below the patient's head. The balance was wirelessly connected to a computer. Using BalanceLink software (Mettler Toledo AG, Greifensee, Switzerland), the time and the weight displayed on the electronic balance was automatically recorded every 5 seconds for the duration of surgery. A 'raw estimate' of urine flow could then be generated automatically by calculating the change in the recorded urine weight every 5 seconds.

Due to the nature of the environment of the operating theatre, the weight of the urine bag could change independently of urine flow. We assessed two different approaches to cleaning the data: 'disturbance-based', and 'inclusion criteria-based'. For the disturbance-based approach, known disturbances to the urine bag or electronic scale, as well as the time at which they occurred, were recorded by the researcher in the operating theatre. These included when: (i) the scale was disturbed, (ii) the urine bag was removed from the scale, (iii) the operating table was moved, (iv) urine samples were taken, (v) the urine catheter bag was emptied, or (vi) the connection between the scale and computer was halted briefly. Such disturbances can produce calculations of negative urine flow, which is not physiologically plausible. The corresponding weight of the urine bag was deleted for each disturbance. The mean urine flow for each epoch was then calculated once all these disturbances were accounted for. For the inclusion criteria-based approach, values of urine flow that fell outside certain ranges were excluded from the data-set.

### **Statistical analyses**

Unless otherwise stated, data are expressed as between subject mean  $\pm$  standard error of the mean. Lines of best fit were generated by OLP regression analysis.<sup>24</sup> Analysis of covariance (ANCOVA) was employed to assess between-subject variability in linear relationships. Both analyses were performed using SYSTAT Version 13 (Systat Inc, San Jose, CA, USA). Univariable logistic regression was used to assess whether thresholds of urinary  $\text{PO}_2$  and/or urine flow during CPB were associated with development of post-operative AKI (SPSS,

Version 23.0, IBM Corporation, Armonk, NY, USA). Two-tailed  $P \leq 0.05$  was considered statistically significant.

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### **Authors' contributions**

Study concept and design: RGE, ADC, JAS, AGT, CNM.

Acquisition, analysis and interpretation of data: MZLZ, AM, JPN, YRL, MK, RGE.

Drafting of the manuscript: JPN, RGE.

Critical revision of the manuscript for intellectual content: JPN, MZLZ, AM, YRL, ADC, JAS, AGT, MK, CNM, RGE.

Obtaining funding: RGE, JAS, ADC, AGT, CNM, YRL.

Statistical analysis: MZLZ, AGT, JPN, YRL, MK.

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### **Conflict of interest statement**

None declared.

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**Table 1.** Regression analysis of the relationship between volumetrically determined and gravimetrically determined urine flow in 20 patients undergoing cardiac surgery requiring cardiopulmonary bypass. Due to the nature of the environment of the operating theatre, negative urine flow was sometimes calculated from the raw 5 s data. Thus the raw 5 s data were subjected to various inclusion criteria.

<b>Data</b>	<b>r<sup>2</sup></b>	<b>P</b>	<b>Slope</b>	<b>Y-intercept</b>	<b>% Data excluded</b>
<b>Raw</b>	<0.001	0.86	2.19 (1.50 to 2.89)	-10.49 (-14.90 to -6.27)	0
<b>Clean; disturbance-based</b>	0.17	<0.001	1.84 (1.39 to -2.29)	-6.96 (-9.71 to -4.21)	9%
<b>Inclusion Criteria (mL min<sup>-1</sup>)</b>					
-20 to 100	0.76	<0.001	1.00 (0.89 to 1.12)	0.42 (-0.28 to 1.11)	12%
-20 to 50	0.67	<0.001	1.05 (0.91 to 1.19)	0.98 (0.11 to 1.85)	13%
-20 to 20	0.82	<0.001	0.78 (0.70 to 0.85)	-0.07 (-0.53 to 0.40)	17%
-10 to 50	0.78	<0.001	1.01 (0.90 to 1.12)	0.35 (-0.32 to 1.01)	14%
0 to 50	0.67	<0.001	1.05 (0.90 to 1.19)	1.04 (0.17 to 1.90)	23%

OLP regression was used to generate lines of best fit in the form of  $y = a + bx$ , where  $x$  is urine flow determined volumetrically,  $y$  is urine flow determined gravimetrically,  $b$  is the slope of the relationship between  $x$  and  $y$ , and  $a$  is the  $y$ -intercept. An optimal method would generate a relationship with  $b = 1$ ,  $a = 0$  and a Pearson product-moment correlation coefficient ( $r^2$ ) = 1.0, while retaining as much of the raw data as possible.  $P$  tests the null hypothesis that the slope of the relationship is zero. The slope and intercept are shown as point estimates and 95% confidence intervals, determined by ordinary least products regression analysis.<sup>24</sup>

**Table 2.** Univariable logistic regression and receiver operator characteristic curve analysis for risk of acute kidney injury associated with various thresholds of urinary oxygen tension and urine flow during cardiopulmonary bypass

Variable	Observations	Odds Ratio	95% CI	<i>P</i>	Sensitivity, %	Specificity, %	AU-ROC (95% CI), <i>P</i>
<b>Urine flow (averaged across the entire period of cardiopulmonary bypass)</b>							
< 3.00 mL min <sup>-1</sup>	19	1.13	0.38 – 3.35	0.82	30.8	71.8	0.51 (0.37 – 0.66), 0.86
< 2.75 mL min <sup>-1</sup>	17	1.48	0.49 – 4.53	0.49	30.8	76.9	0.54 (0.39 – 0.68), 0.60
< 2.50 mL min <sup>-1</sup>	17	1.48	0.49 – 4.53	0.49	30.8	76.9	0.54 (0.39 – 0.68), 0.60
< 2.25 mL min <sup>-1</sup>	15	2.03	0.63 – 6.53	0.23	30.8	82.1	0.55 (0.41 – 0.70), 0.42
< 2.00 mL min <sup>-1</sup>	10	2.63	0.66 – 10.4	0.17	23.1	89.7	0.56 (0.42 – 0.71), 0.38
< 1.75 mL min <sup>-1</sup>	7	2.18	0.45 – 10.7	0.34	15.4	92.3	0.54 (0.39 – 0.68), 0.60
< 1.50 mL min <sup>-1</sup>	6	1.57	0.29 – 8.43	0.60	11.5	92.3	0.52 (0.37 – 0.66), 0.79
< 1.25 mL min <sup>-1</sup>	5	1.00	0.16 – 6.44	1.00	0.0	100	0.50 (0.36 – 0.64), >0.99
< 1.00 mL min <sup>-1</sup>	1	-	-	-	-	-	-
<b>Urinary PO<sub>2</sub> (measured continuously, so the threshold was met if it fell below this level at any time during cardiopulmonary bypass)</b>							
< 15 mmHg	43	1.26	0.44 – 3.63	0.67	0.0	100	0.52 (0.38 – 0.67), 0.73
< 12.5 mmHg	29	2.44	0.88 – 6.73	0.09	57.7	64.1	0.61 (0.47 – 0.75), 0.14
< 10 mmHg	24	4.54	1.55 – 13.3	0.006	57.5	76.9	0.67 (0.54 – 0.81), 0.02
< 7.5 mmHg	15	6.42	1.76 – 23.4	0.005	42.3	89.7	0.66 (0.52 – 0.80), 0.03
< 5.0 mmHg	9	6.82	1.29 – 36.1	0.02	26.9	94.9	0.61 (0.46 – 0.75), 0.14
<b>Composite criterion</b>							
Urinary PO <sub>2</sub> < 10 mmHg or urine flow < 2 mL	29	5.73	1.93 – 17.0	0.002	69.2	71.8	0.71 (0.57 – 0.84), 0.005

min<sup>-1</sup>

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These analyses are based on the dataset of 65 patients, 26 of whom developed post-operative acute kidney injury, reported by Zhu et al.<sup>4</sup> In the original report urine flow and urinary PO<sub>2</sub> were considered across the entire surgical procedure.<sup>4</sup> In the current analysis we only consider these variables during the period of cardiopulmonary bypass. The term ‘Observations’ refers to the number of patients who met each of the stated criteria.

Abbreviations: UPO<sub>2</sub>, urinary oxygen tension; CI, confidence interval; AU-ROC, area under the receiver operator characteristic curve.

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### Figure Legends

**FIGURE 1. Scatterplots of (A) medullary PO<sub>2</sub>, (B) urine flow and (C) arterial PO<sub>2</sub> vs urinary PO<sub>2</sub> in sheep.** Each color represents a different treatment group. Lines of best fit were generated by ordinary least products regression.<sup>24</sup> The line of best fit for **(A)** is  $y = 1.03x + 1.85$ , ( $r^2_1 = 0.29$ ,  $P < 0.001$ ;  $r^2_2 = 0.56$ ,  $P < 0.001$ ), **(B)** is  $y = 14.72x + 16.17$  ( $r^2_1 = 0.03$ ,  $P < 0.001$ ;  $r^2_2 = 0.47$ ,  $P < 0.001$ ), and **(C)** is  $y = 1.57x - 104.26$  ( $r^2_1 = 0.06$ ,  $P < 0.001$ ;  $r^2_2 = 0.50$ ,  $P < 0.001$ ). The variable  $r^2_1$  is the Pearson product-moment correlation coefficient for the linear relationship between the continuous independent variable (medullary PO<sub>2</sub>, arterial PO<sub>2</sub> or urine flow) and urinary PO<sub>2</sub>. The variable  $r^2_2$  also includes the categorical variable ‘sheep’ and the interaction term, between sheep and the continuous independent variable, in the model. Saline (n=9; black), NA = noradrenaline (n=5; blue), Vol Res = volume resuscitation (n=6; red), ANG II = angiotensin II (n=8; green).

**FIGURE 2. Scatterplots of urine flow and arterial PO<sub>2</sub> versus the difference between medullary PO<sub>2</sub> and urinary PO<sub>2</sub> in sheep.** Each color represents a different treatment group. Lines of best fit were generated by ordinary least products regression.<sup>24</sup> The line of best fit for **(A)** is  $y = 13.78x - 12.59$ , ( $r^2_1 = 0.01$ ,  $P = 0.005$ ;  $r^2_2 = 0.42$ ,  $P < 0.001$ ). For **(B)**  $r^2_1 = 0.003$ ,  $P = 0.47$ ;  $r^2_2 = 0.10$ ,  $P < 0.001$ . The variable  $r^2_1$  is the Pearson product-moment correlation coefficient for the linear relationship between the continuous independent variable (arterial PO<sub>2</sub> or urine flow) and the difference between medullary and urinary PO<sub>2</sub>. The variable  $r^2_2$  also includes the categorical variable ‘sheep’ and the interaction term, between sheep and the continuous independent variable, in the model. Saline (n=9; black), NA = noradrenaline (n=5; blue), Vol Res = volume resuscitation (n=6; red), ANG II = angiotensin II (n=8; green).

**FIGURE 3. Optimization of data cleaning methods for gravimetric determination of urine flow in 20 patients undergoing cardiac surgery requiring cardiopulmonary bypass.** The raw 5 s data obtained gravimetrically were first subjected to five different inclusion criteria: **(A)** -20 to 100 mL min<sup>-1</sup>, **(B)** -20 to 20 mL min<sup>-1</sup>, **(C)** -20 to 50 mL min<sup>-1</sup>, **(D)** -10 to 50 mL min<sup>-1</sup>, and **(E)** 0 to 50 mL min<sup>-1</sup>. The mean urine flow was then calculated for each of the four epochs for both the volumetric and gravimetric approaches. Each point represents the co-ordinate for the volumetrically determined urine flow versus the gravimetrically determined urine flow for a single epoch of surgery for an individual patient.

Lines of best fit were determined by ordinary least products regression.<sup>24</sup> Regression coefficients are shown in Table 1.

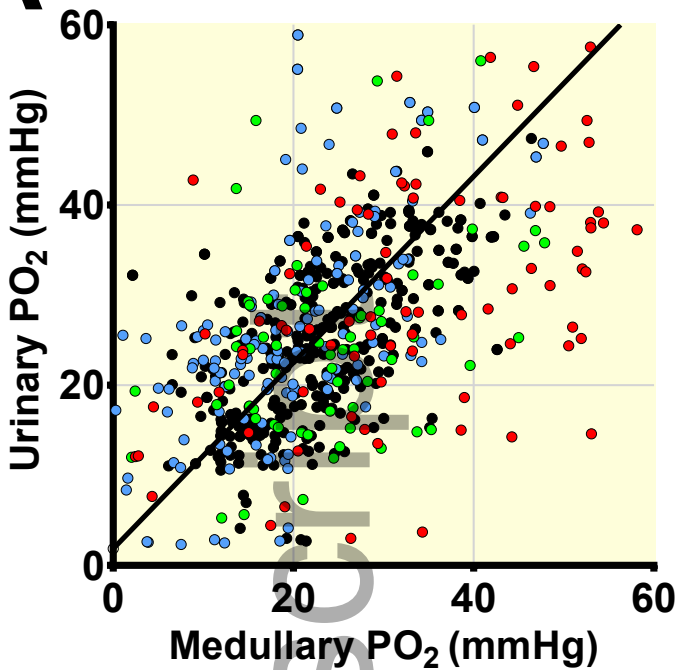
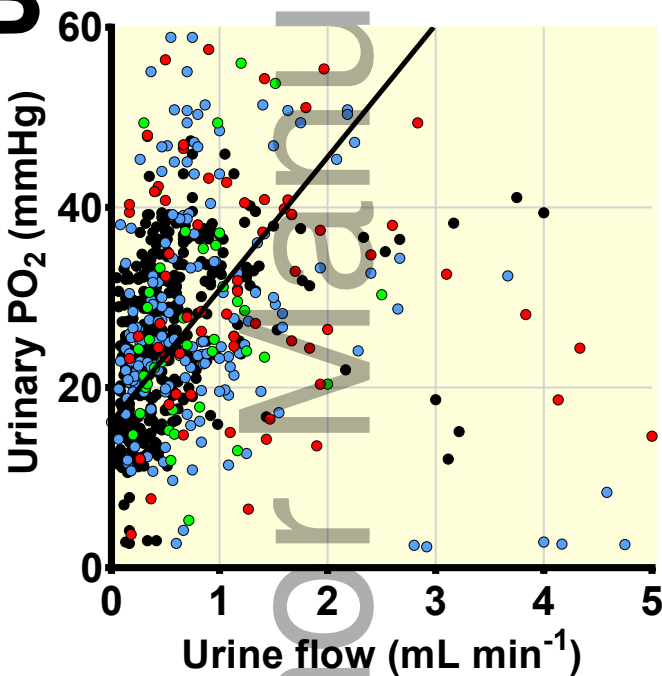
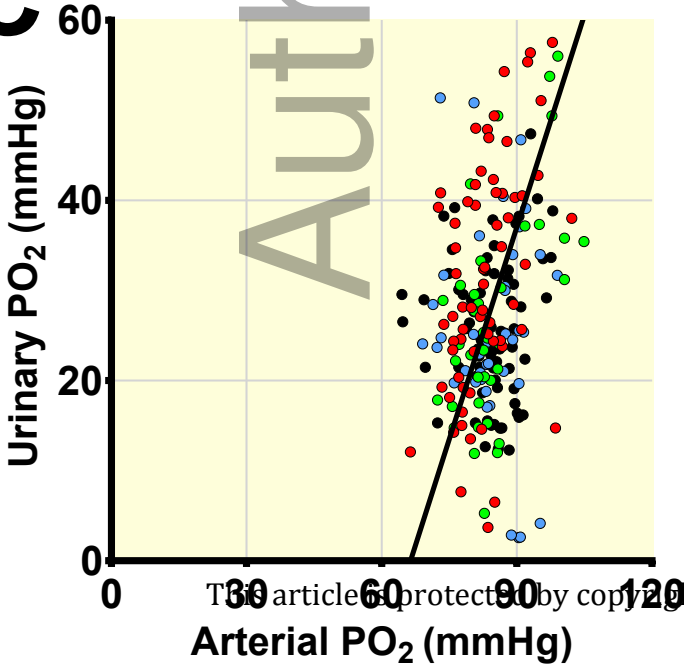
**FIGURE 4. Temporally resolved urine flow during cardiac surgery requiring cardiopulmonary bypass.** Representative data are shown for 6 of the 20 patients included in the analysis. Raw 5 s estimates of urine flow, after exclusion of values  $< -10 \text{ mL min}^{-1}$  or  $> 50 \text{ mL min}^{-1}$ , are shown in green. One-minute averages of these data are shown in red and 5-minute averages are shown in yellow. Vertical dotted lines show the transition between the various epochs of the surgical procedure. **(A-C)** Patients who did not develop AKI and **(D-F)** patients who did develop AKI.

**FIGURE 5. Temporal relationships between urinary  $\text{PO}_2$ , urine flow, and arterial  $\text{PO}_2$  during cardiac surgery requiring cardiopulmonary bypass (CPB).** Lines represent urinary  $\text{PO}_2$  (blue), arterial  $\text{PO}_2$  (red) and gravimetrically determined urine flow (black) determined at 5-min intervals. Arterial  $\text{PO}_2$  could only be measured during CPB, using an in-line blood gas analyser. Vertical dotted lines show the transition between the various epochs of the surgical procedure. **(A-C)** Patients who did not develop AKI and **(D-F)** patients who did develop AKI.

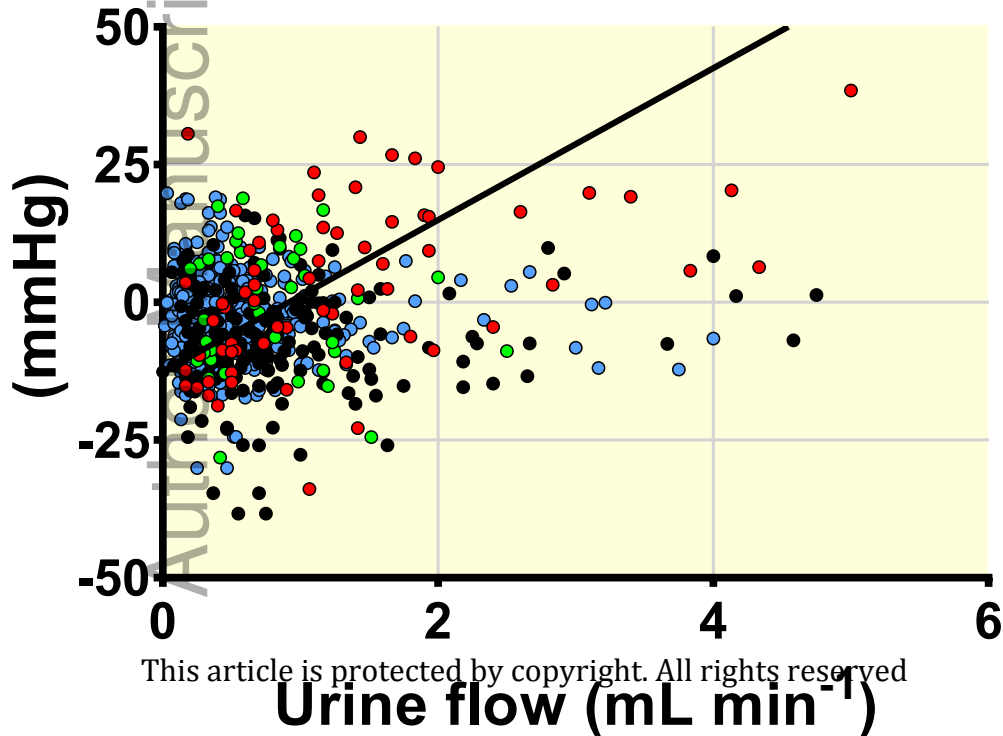
**FIGURE 6. Arterial  $\text{PO}_2$  and urine flow vs urinary  $\text{PO}_2$  during bypass.** Urinary  $\text{PO}_2$  and arterial  $\text{PO}_2$  were taken at 5-min intervals. Urine flow was taken from the calculated 5-minute averages. Each color represents a different patient, total 20 patients. Lines of best fit were generated by ordinary least products regression (line not shown).<sup>24</sup> **(A)**  $y = -4.19x + 57.69$ , ( $r^2 = 0.16$ ,  $P < 0.001$ ) and **(B)**  $r^2 = 0.004$ ,  $P = 0.15$ .

**A**

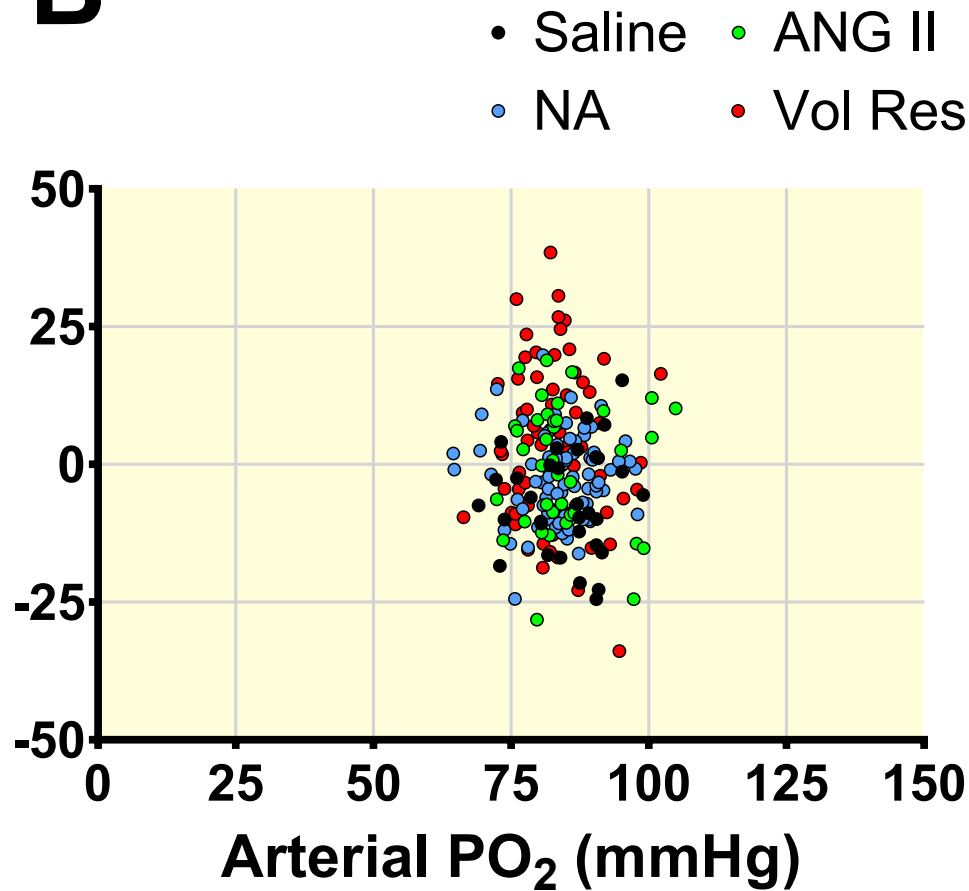
• Saline • ANG II  
• NA • Vol Res

**B****C**

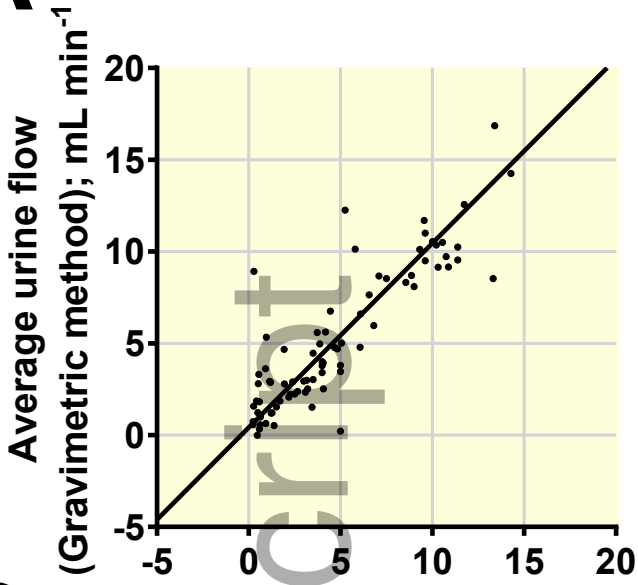
**A**



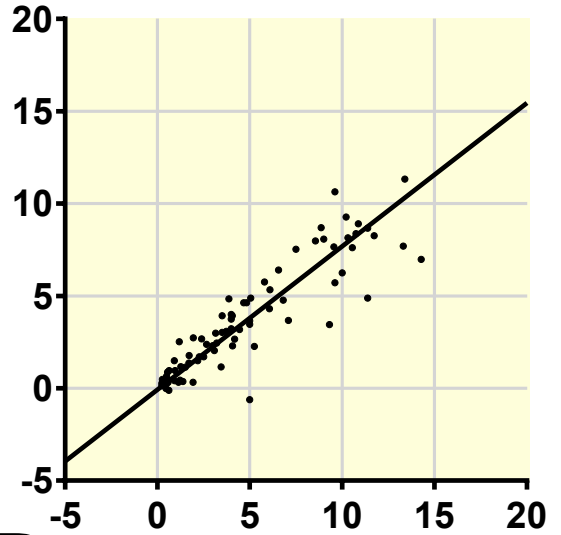
**B**



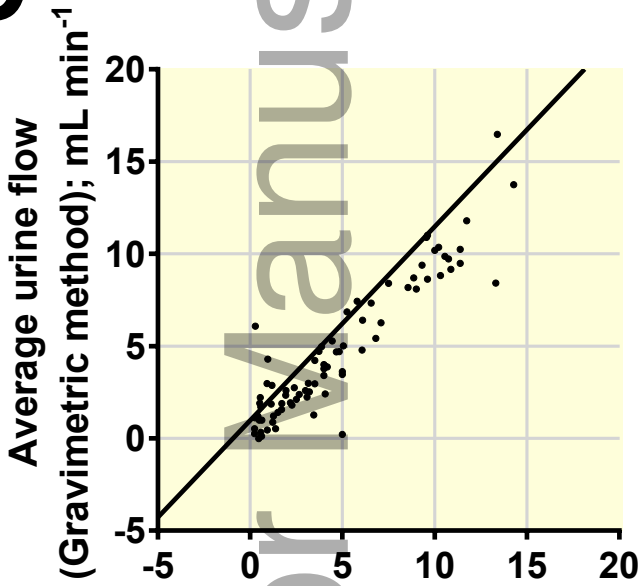
**A**



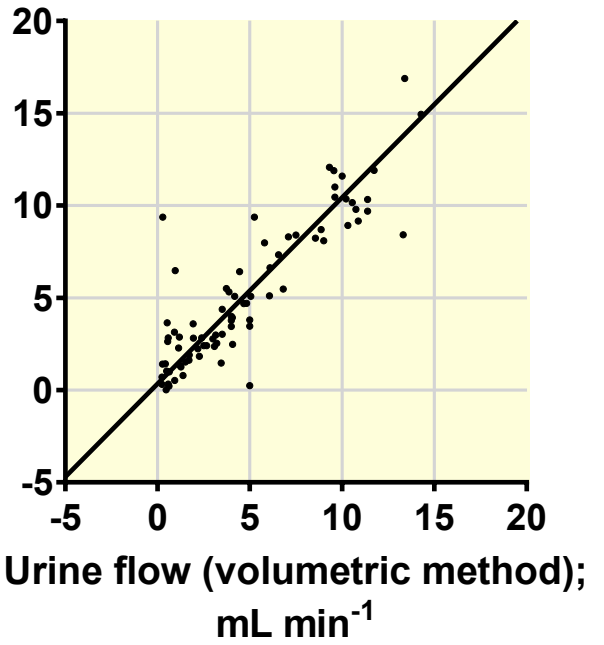
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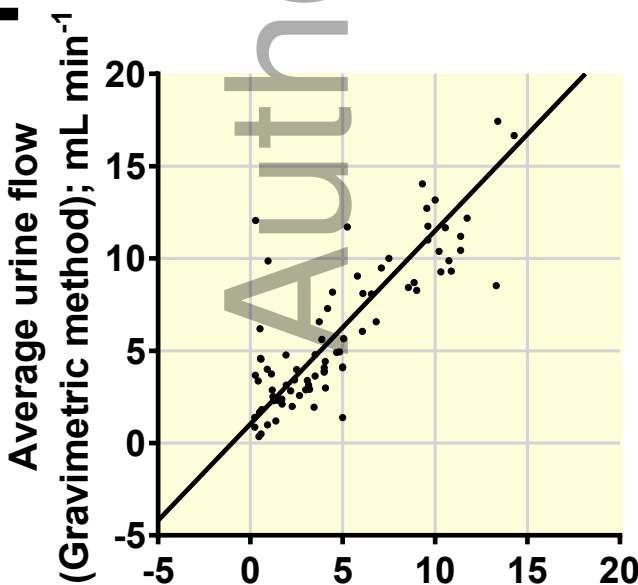
**C**

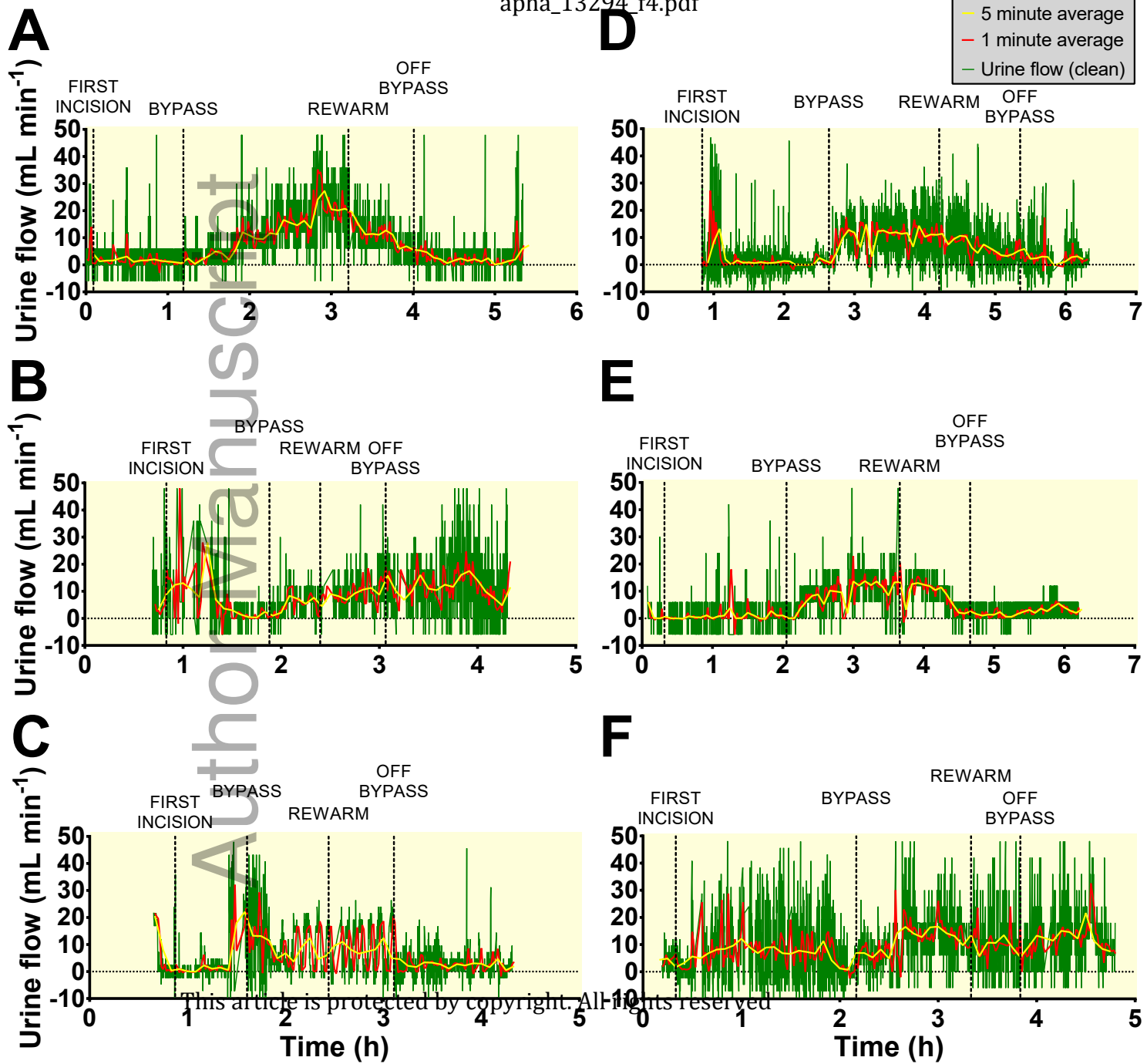


**D**

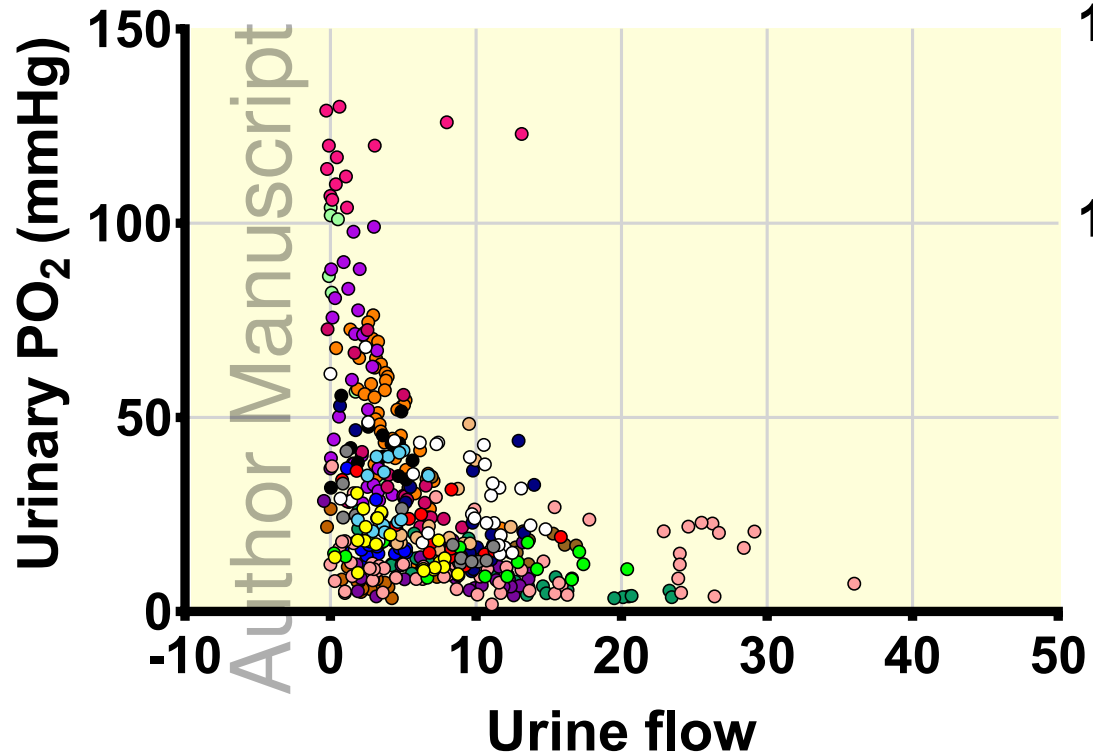


**E**





**A**



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**( $mL\ min^{-1}$ ; 5-min averages)**

**B**

