

# A technique for the simultaneous measurement of renal ATP, blood flow and pH in a large animal model of septic shock

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Acute renal failure (ARF) is relatively common in critically ill patients and carries a high mortality.<sup>1</sup> Sepsis is the most common cause of ARF in the intensive care unit,<sup>2</sup> but understanding of its pathogenesis is limited. Most animal models of ARF have limited relevance to human septic ARF,<sup>3</sup> because they are based on ischaemia. These models have led to the paradigm that medullary ischaemia might be responsible for ARF.<sup>4</sup> However, models of hyperdynamic sepsis fail to show either decreased global renal blood flow<sup>5</sup> or decreased medullary flow,<sup>6</sup> suggesting that the pathogenesis of ARF during hyperdynamic sepsis might be different. On the other hand, cellular (histotoxic) hypoxia might yet occur in sepsis. In-vivo measurement of renal ATP and renal pH during hyperdynamic sepsis should help confirm or exclude the presence of such cellular hypoxia. This is theoretically possible with <sup>31</sup>P magnetic resonance spectroscopy<sup>7</sup> and has been performed in transplanted kidneys and small animals.<sup>7,8</sup> However, in small animals, it is currently extremely difficult to continuously monitor arterial blood pressure and renal blood flow. This simultaneous monitoring is needed to understand the relationship between blood pressure, global renal blood flow, intrarenal ATP and pH. This monitoring is theoretically possible in a large animal. We recently obtained sequential <sup>31</sup>P magnetic resonance spectra over long periods of time from native kidneys in a sheep with septic shock.<sup>9</sup> However, due to the effect of the magnet on the flow meter, we were unable to obtain simultaneous data on renal blood flow.

Accordingly, we conducted a further study:

- to develop a technique to simultaneously measure mean arterial pressure (MAP), renal blood flow, renal ATP and renal pH in a large mammal during severe sepsis and circulatory arrest; and
- to describe preliminary observations during such measurements.

## Methods

All experimental procedures were approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute of the University of Melbourne, VIC, under guidelines laid down by the National Health and Medical Research Council of Australia.

## ABSTRACT

**Background:** Simultaneous measurement of renal blood flow, renal ATP, renal pH and mean arterial pressure (MAP) might help investigators understand the mechanisms responsible for acute renal failure (ARF) in sepsis.

**Objectives:** (1) To develop a technique to simultaneously measure MAP, renal blood flow, renal ATP and renal pH in a large mammal during severe sepsis and after circulatory arrest; and (2) To describe preliminary observations during such measurements.

**Methods:** We implanted a custom-made phosphorus coil around the left kidney and a magnetic resonance-compatible blood flow probe around the renal artery of an adult Merino ewe. We induced severe sepsis by intravenous administration of *Escherichia coli* and obtained <sup>31</sup>P magnetic resonance spectroscopic data at 3tesla, and continuous blood flow and MAP data before and during severe sepsis over several hours. We induced circulatory arrest with potassium chloride and measured the same <sup>31</sup>P signal immediately and again 30 minutes later.

**Results:** We successfully and simultaneously measured MAP, renal blood flow, renal ATP and renal pH in a large mammal during severe sepsis and induced circulatory arrest. With these techniques, we observed that, despite marked hypotension, there were limited changes in renal ATP and renal pH, and that renal blood flow increased. A rapid and dramatic decrease in ATP and pH occurred with circulatory arrest.

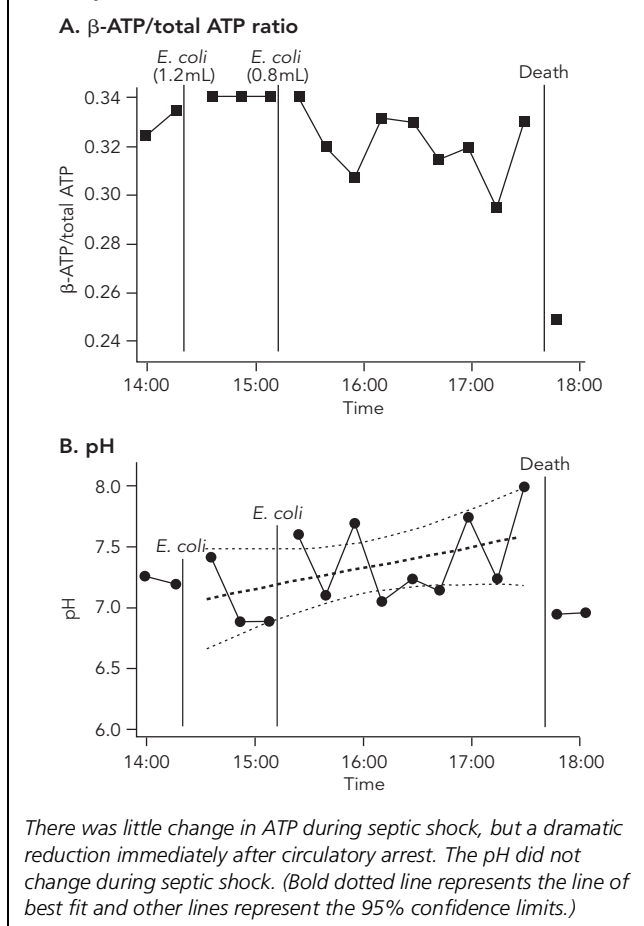
**Conclusions:** We have developed a technique to simultaneously monitor MAP, renal blood flow, ATP and pH in a large mammal during severe sepsis. Our initial observations indicate preservation of renal ATP in septic shock.

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## Animal preparation

Under anaesthesia, using aseptic procedures, a phosphate coil was placed around the left kidney of an adult Merino ewe (38kg) to allow measurement of renal ATP. The implantable coil was custom-made in-house and comprised a single-tuned phosphorus (<sup>31</sup>P) match+tune+tune-balance variable capacitor network using a Helmholtz transmit-receive coil.

**Figure 1.  $\beta$ -ATP/total ATP ratio and pH peak value in a sheep with septic shock induced by *Escherichia coli* injection**



Each loop in the coil was about 40mm in diameter, and the interloop bridge distance was about 25mm, allowing the coil to sit snugly around the main body of the kidney without causing superficial ischaemia. Two flexible leads of about 200mm length were used to connect the coil to the external capacitor network. In-situ tuning was achieved using a Wavetek frequency sweep generator (Wavetek, San Diego, Calif, USA).

Anaesthesia for coil implantation was induced with intravenous sodium thiopentone (15mg/kg) and, following intubation, was maintained with 1.5%–2.0% isoflurane in a mixture of medical grade air and O<sub>2</sub>. A paracostal retroperitoneal approach was used to expose the kidney. The coil was placed around the kidney, and the cables from the coil were brought out through the skin on the dorsal flank. At the time of surgery for coil implantation, a magnet-compatible Doppler flow probe (Transonics Systems, Ithaca, NY, USA) was inserted around the left renal artery. In addition, a carotid artery was exposed in the neck, and a Tygon

catheter (internal diameter, 1.0mm, outside diameter, 1.7mm; Cole-Parmer, Bona, VIC) filled with heparinised saline (100IU/mL) was inserted 15–20cm towards the heart. The skin was sutured, and the catheter was externalised.

Antibiotics (0.4g procaine benzylpenicillin and 0.5g dihydrostreptomycin sulfate; Norbrook Laboratories, UK) were administered prophylactically for 3 days after surgery. A week after surgery, correct position of the coil on the kidney was confirmed fluoroscopically (a 1.0cm length of radio-opaque suture had been glued to the renal capsule during the surgical procedure to enable the position of the kidney to be visualised).

Two days before experimentation, a jugular venous triple-lumen catheter (cross section, 7Fr; 2.4mm) (ARROW International, Reading, Pa, USA) was placed for the measurement of central venous pressure and for infusion.

### Experimental protocol and measurements

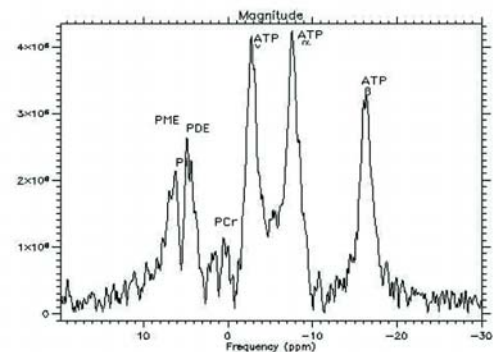
On the day of the experiment, anaesthesia was induced with sodium thiopentone (15mg/kg) for endotracheal tube placement (cuffed size, 10), followed by insertion of a urinary catheter. Anaesthesia was maintained with a mixture of oxygen, air and isoflurane (2%–3%). Fractional inspired oxygen was altered to maintain PaO<sub>2</sub> at about 100mmHg, and ventilation was controlled to maintain PaCO<sub>2</sub> at about 40mmHg. Arterial pressure and central venous pressure (CVP) were measured continuously using a PowerLab digital acquisition system (ADInstruments, Sydney, NSW) and recorded on a Powerbook computer (Apple Computers, Calif, USA). The renal flow probe was connected to a flow meter (T201 CDS; Transonics Systems, Ithaca, NY, USA), and renal blood flow was simultaneously measured continuously. The flow meter was located outside the magnet room to attenuate magnetic interference.

Following a 2-hour observation period, the animal was injected intravenously with 1.2mL of a suspension of viable *Escherichia coli* ( $3 \times 10^9$  colony forming units in 50mL normal saline) delivered over 5 minutes. This was followed 40 minutes later by a second injection of 0.8mL. Shock was defined by a MAP < 60mmHg. The sheep became hypotensive within 30 minutes of the second injection, with an MAP of about 45mmHg. Fluid resuscitation was then performed with 1.5L Gelofusine (B. Braun, Melsungen, Germany) followed by 100mL/h intravenous fluid maintenance to keep MAP above 50mmHg. Urine output in this hour was about 15mL. One hour after *E. coli* injection, the MAP was allowed to decrease to 45–50mmHg and to remain at this level until the animal was given a lethal dose of potassium chloride. The period of observation was chosen to reproduce the duration of previous experiments on septic shock in sheep.<sup>10</sup>

Circulatory arrest was induced with potassium chloride and the animal was kept in the magnetic scanner for

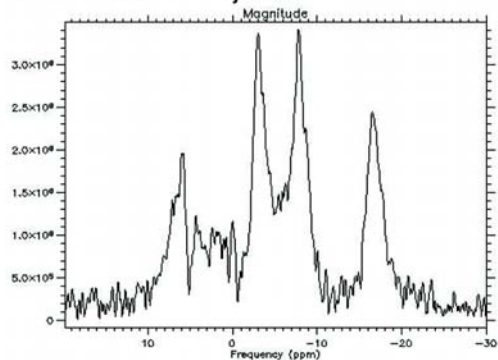
**Figure 2.**  $^{31}\text{P}$  magnetic resonance spectra in a sheep with septic shock induced by *Escherichia coli* injection

**A. Baseline**



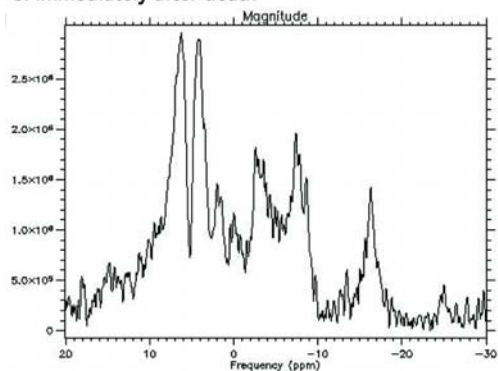
Baseline spectrum showing the major  $^{31}\text{P}$  metabolites: phosphomonoesters (PME), phosphodiesters (PDE), phosphocreatine (PCr), and the three phosphates of ATP ( $\alpha$ -ATP,  $\beta$ -ATP and  $\gamma$ -ATP). The inorganic phosphate (Pi) signal is hidden within the PDE peak (before cardiac arrest the PDE-Pi peak is mostly due to PDE, while after cardiac arrest, Pi predominates).

**B. 2 hours after *E. coli* injection**



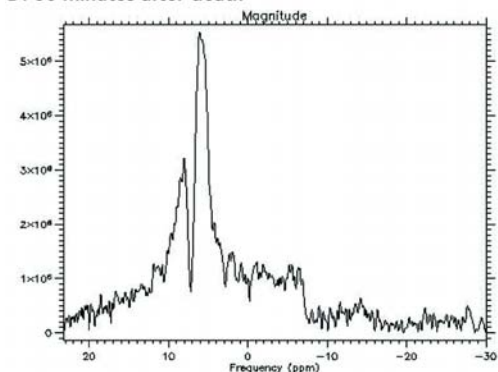
2 hours after injection of *Escherichia coli*, ATP showed little change.

**C. Immediately after death**



Immediately after a lethal dose of potassium chloride, ATP peaks were low relative to phosphomonoesters and inorganic phosphate, which was then mostly responsible for the PDE-Pi peak.

**D. 30 minutes after death**



30 minutes after a lethal dose of potassium chloride, ATP peaks were absent, and the inorganic phosphate peak dominated.

another 30 minutes for further measurements. The position of the coil on the kidney was confirmed at autopsy.

$^{31}\text{P}$  spectra were recorded with the implanted Helmholtz coil every 15 minutes for the duration of the study. Data were acquired at 51.705 MHz in a 3.0 tesla magnetic resonance scanner (LX Horizon, GE Medical Systems, Wis, USA) using a flip angle of  $90^\circ$  and a relaxation delay of 2 s. Spectra were recorded from a 10 cm thick slice using a spin-echo sequence and represent an average of 450 transients of 2048 data points acquired over a spectral width of 16 000 Hz. Total acquisition time was 15 minutes. Respiratory gating was not used.

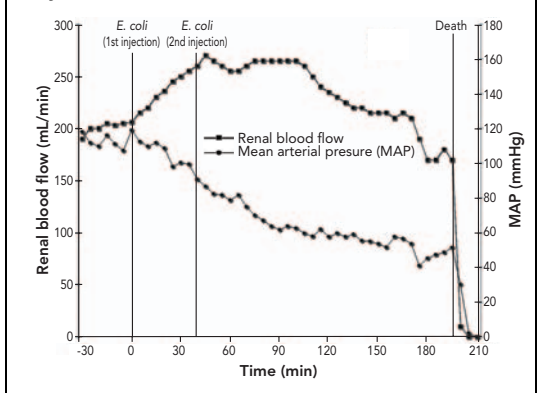
Spectra were processed using SAGE software (GE Medical Systems). Data were zero filled twice, and a 10 Hz Lorentzian apodization function was applied before Fourier transformation. The peak at lower frequency adjacent to the  $\gamma$ -ATP signal was assumed to be phosphocreatine and referenced to 0.0 ppm. Peak areas were determined using the integration routine of SAGE, and renal parenchymal pH was calculated.

**Results**

Shock was achieved in the sheep, with onset of significant hypotension by 30 minutes after the administration of two boluses of *E. coli*.

We successfully obtained  $^{31}\text{P}$  spectra at baseline, during sustained septic shock and following death, while simultaneously measuring renal pH, renal blood flow with a magnetic resonance-compatible flow probe, and MAP. We found no

**Figure 3.** Mean arterial pressure (MAP) and renal blood flow in a sheep with septic shock induced by *Escherichia coli* injection



change in ATP signal during septic shock compared with pre-induction levels, despite a significant decrease in blood pressure. Similarly to overall ATP, the ratio of  $\beta$ -ATP/total ATP showed only minor change, while renal pH did not change significantly during severe sepsis (Figure 1).  $^{31}\text{P}$  spectra are shown in Figure 2.

Finally, despite marked hypotension, renal blood flow increased during septic shock (Figure 3).

There was a predictable loss of ATP and renal blood flow signal at death (Figure 2D and Figure 1).

## Discussion

We conducted a complex experiment to test whether we could develop a technique to simultaneously measure MAP, renal blood flow, renal ATP and renal pH in a large mammal during severe sepsis and circulatory arrest, and to describe preliminary observations obtained during such measurements.

We found that we could successfully measure all of the above variables simultaneously. We also found that septic shock induced by *E. coli* was associated with increased renal blood flow, and that renal ATP remained essentially unchanged for the duration of septic shock. Finally, we found full loss of ATP signal and renal blood flow signal at death, as expected.

In our previous study,<sup>9</sup> we reported the successful acquisition of high-quality spectra in a reproducible, sequential manner, allowing monitoring over several hours. However, we were unable to relate these findings to continuous measurements of renal blood flow and blood pressure, because the magnetic scanner interfered with these measurements. In this experiment, we successfully implanted a magnet-compatible flow probe, which was not displaced during spectra acquisition and whose signal could be obtained reliably through a flow meter placed outside the sealed magnet room. We were similarly able to obtain continuous information on MAP by placing transducers and monitor outside the magnet room. These technical modifications achieved continuous monitoring of renal blood flow and arterial pressure, permitting continuous assessment of hypotension. To our knowledge, this is the first time that such simultaneous measurements have been reported in a large animal.

Even in a single animal, these technical developments provided interesting observations, with very limited changes in renal ATP concentration and renal pH despite severe hypotension. This pattern differed completely from that induced by ischaemia (circulatory arrest). We also observed increased renal blood flow despite hypotension, indicating significant vasodilatation of the renal vasculature. These preliminary observations already provide proof of concept that, early in septic shock, severe hypotensive bacteraemia may be associated with renal vasodilatation, and that such severe hypotension does not necessarily induce ATP depletion.

This technique represents an important step forward in our ability to monitor kidney function, renal blood flow, acid-base status and bioenergetic balance simultaneously. It opens the door to extended and randomised, controlled experimental studies to more accurately define changes in these variables during experimental septic shock.

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