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# Changes in kidney perfusion and renal cortex metabolism in septic shock: an experimental study

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ARTICLE INFO

*Article history:*
Received 19 May 2016
Received in revised form
25 July 2016
Accepted 24 August 2016
Available online 2 September 2016

*Keywords:*
Kidney injury
Renal blood flow
Sepsis
Lactate
Pyruvate

ABSTRACT

*Background:* The etiology of renal dysfunction in sepsis is currently attributed to altered perfusion, microcirculatory abnormalities and cellular alterations. To clarify these mechanisms, we characterized the changes in renal perfusion and cortex metabolism in a large animal model of sepsis.

*Methods:* We studied 12 adult female sheep randomized to peritonitis-induced sepsis ($n = 8$) or to sham procedure ($n = 4$). A flow probe was positioned around the renal artery to measure renal blood flow (RBF). Laser Doppler was used to measure regional flow in the kidney cortex and medulla. A microdialysis probe was inserted into the renal cortex to measure cortical glucose, lactate, and pyruvate. Fluid resuscitation was provided to keep pulmonary artery occlusion pressure at baseline levels. All animals were observed for 18 h.

*Results:* Hypotension occurred after 9 h in the septic animals ($P = 0.02$ *versus* baseline). RBF and cortical flow were significantly lower than at baseline from 12 h in the septic animals ($P = 0.01$ and $P = 0.03$, respectively). Cortical lactate and pyruvate levels increased in the septic animals from 3 and from 6 h, respectively (both $P = 0.02$ *versus* baseline), and the L/P ratio from 15 h ($P = 0.01$). There was a correlation between cortical flow and cortical L/P ratio after shock onset ($r = -0.60$, $P = 0.002$) but not before.

*Conclusions:* In this peritonitis model, sepsis was associated with metabolic alterations that may reflect early induction of cortical glycolysis. Septic shock was associated with reduced renal perfusion and decreased cortical and medullary blood flow, followed by signs of anaerobic metabolism in the cortex when flow reductions became critical.



## Introduction

Sepsis and septic shock are a major burden to health care systems worldwide.[1] Acute kidney injury (AKI) is a frequent complication of sepsis and contributes independently to sepsis-related morbidity and mortality.[2-4] Although our understanding of the underlying pathogenesis of AKI in this setting is still limited, reduced renal perfusion and

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0022-4804/$ – see front matter 
http://dx.doi.org/10.1016/j.jss.2016.08.083

microcirculatory alterations are likely to be involved.[5,6] Macrocirculatory and microcirculatory perfusion deficits are ubiquitous in the septic kidney and often precede or coincide with the occurrence of renal dysfunction.[7-9] Microvascular hypoperfusion is associated with localized areas of hypoxia that may be, at least partially, corrected by fluid resuscitation.[5,10] This finding supports an important role of perfusion alterations in septic AKI, but it is still unknown as to whether these alterations are sufficiently severe to cause actual deficits in cellular oxygen.

Tubular epithelial cells located adjacent to no-flow capillary vessels have been found to have an altered redox status, suggesting the presence of anaerobic metabolism due to reduced oxygen delivery.[6,7] However, histologic findings in septic AKI are usually not consistent with signs of widespread necrotic or apoptotic cell death.[11,12] Moreover, some investigators have found that sepsis may be associated with renal hyperemia, suggesting a secondary, if any, role for hypoxia in sepsis-associated AKI.[13,14]

In addition to the controversy surrounding cellular oxygenation, data on renal metabolism during sepsis are also scarce and contradictory. Because of the invasive nature of its assessment, no data are available on renal metabolism in human sepsis and septic shock. However, in a canine model of endotoxemia, the kidney appeared to be a net consumer of lactate.[15] Conversely, Benes *et al.* showed in septic pigs that renal hypoperfusion was associated with an increase in the lactate/pyruvate (L/P) ratio in the renal vein, suggesting the presence of renal lactate production.[16] In a mouse model of ischemia-reperfusion injury, Zager *et al.* demonstrated that increased anaerobic glycolysis and subsequent pyruvate deprivation were important pathways leading to renal dysfunction.[17] Moreover, Smith *et al.* reported an increased expression of activated hexokinase—a key-regulator of glycolysis—in the renal cortex of endotoxemic mice.[18] These findings indicate that renal cortex metabolism, in particular glycolysis, may be altered in ischemic conditions, although the effect of sepsis on renal metabolism is largely undefined.

Given the uncertainty about the relationship between impaired kidney perfusion and metabolism, we designed a study to characterize the evolution of renal flow alterations and kidney cortex metabolism in a clinically relevant large animal model of sepsis and septic shock.

## Materials and methods

We studied 12 adult female sheep (aged, 8-10 mo; weight, 26 [24-32] kg). In this ovine model, shock and multiple organ failure develop gradually, closely mimicking the clinical findings in sepsis and septic shock. Moreover, the size of the animals enables continuous hemodynamic monitoring and endpoint-oriented resuscitation to be achieved, which further enhances the external validity of the model. The animals were fasted for 18 hours before the start of the experiment with free access to water. The local ethics committee of the Free University of Brussels, Belgium, approved the study, and care and handling of the animals were in accordance with National Institute of Health Guidelines.

### *Anesthesia and ventilation*

On the day of the experiment, the animals were weighed and premedicated with a mixture of 0.5 mg/kg midazolam (Dormicum; Roche SA, Anderlecht, Belgium) and 40 mg/kg ketamine hydrochloride (Imalgine; Merial, Lyon, France). After the animals had been placed in the supine position, a 14G peripheral cannula (Terumo, Leuven, Belgium) was inserted into the cephalic vein for endovascular access. The animals were intubated using an 8.0 mm diameter endotracheal tube (Mallinckrodt Medical, Dublin, Ireland) following an intravenous (i.v.) bolus of 30 μg/kg fentanyl citrate (Janssen Pharmaceutica, Beerse, Belgium) and 0.5 mg/kg of rocuronium bromide (Esmeron; Organon, Oss, The Netherlands). All animals were sedated with a continuous infusion of midazolam at 1.5 mg/kg/h and ketamine hydrochloride at 10 mg/kg/h. Morphine (1.0 mg/kg/h) was given for analgesia, and muscular blockade was achieved by administration of rocuronium at a rate of 0.1 mg/kg/h. Mechanical ventilation (Servo300 ventilator; Siemens-Elema, Solna, Sweden) was performed in volume-controlled mode, with a tidal volume of 10 mL/kg, a positive end-expiratory pressure of 5 cm $H_2O$ and a fraction of inspired oxygen ($FiO_2$) of 30%. $FiO_2$ was adjusted to keep $PaO_2$ >80 mm Hg. A 60 cm tube was inserted into the stomach to drain its content and prevent rumen distension. A 14-F Foley catheter (Beiersdorf AG, Hamburg, Germany) was introduced into the bladder to monitor urine output.

### *Surgical preparation*

We exposed the right common carotid artery and introduced a 4.5-F arterial catheter (Vygon, Cirencester, England) that was connected to a pressure transducer (True Wave; Edwards LifeSciences, Irvine, CA). A 7-F introducer was inserted into the surgically exposed external jugular vein and a pulmonary artery catheter (Edwards LifeSciences) advanced under monitoring of pressure waveforms.

Animals were then randomized to sepsis ($n = 8$) or sham procedure ($n = 4$). In animals randomized to the sepsis group, a midline laparotomy was performed. After cecotomy, 1.5 g/kg body weight of feces was collected. The cecum was closed and returned to the abdominal cavity. A 25-cm tube was left in the abdominal cavity for feces injection. If the animal was randomized to the sham procedure, a skin incision was made but the abdomen was not opened.

After abdominal surgery, all animals were turned to the prone position. In both groups, the left renal artery was surgically exposed following a flank incision, and a precalibrated flow probe (PS series 6 mm; Transonic, Ithica) was positioned around it. A 3-F catheter (Vygon) was introduced into the left renal vein using the Seldinger technique to enable blood sampling.

The kidney fascia was punctured, and a microdialysis catheter (membrane length 4 mm, cutoff 20 kDa; CMA 20; Microdialysis, Solna, Sweden) introduced into the renal cortex. A second puncture enabled insertion of a laser Doppler probe (OxyFlo; Oxford Optronix, Oxford, United Kingdom) for monitoring of the cortical circulation. A second laser Doppler probe was advanced into the medulla.