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# Sepsis results in an altered renal metabolic and osmolyte profile

*Paul Waltz, MD,*[a] *Evie Carchman, MD,*[a] *Hernando Gomez, MD,*[b] *and Brian Zuckerbraun, MD*[a,c,*]

[a] *Department of Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania*
[b] *Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania*
[c] *VA Pittsburgh Healthcare System, University Dr C, Pittsburgh, Pennsylvania*

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ABSTRACT

*Background:* Sepsis remains a major health-care burden and source of morbidity and mortality. Acute kidney injury and failure frequently accompanies severe sepsis and contributes to this burden. Despite a great deal of research, the exact mechanisms underlying renal failure in sepsis are poorly understood. This study aims to further understand metabolic changes in renal tissue during sepsis.

*Materials and methods:* Experimental sepsis was induced by cecal ligation and puncture (CLP) in C57BL/6 mice. Serum and organs were harvested 8 h after CLP. Markers of renal function including serum creatinine, blood urea nitrogen, and cystatin C were measured. Whole kidneys were analyzed for a global biochemical profile via liquid chromatography/tandem mass spectrometry by Metabolon.

*Results:* CLP induced renal injury as evidenced by elevated serum creatinine, blood urea nitrogen, and cystatin C. Global energetic profile in sepsis showed an increase in glycolytic intermediates with decreased flux through the tricarboxylic acid (TCA) cycle. Multiple inflammatory markers were elevated in response to CLP. Levels of osmotic regulators varied, with an overall increase in pinitol, urea, and taurine in response to CLP.

*Conclusions:* CLP resulted in dramatic changes in the renal macromolecular milieu. There appears to be an increased dependence on glycolysis and diminished flush through the TCA cycle. In addition, changes in renal osmolytes including pinitol, urea, and taurine were observed, perhaps uncovering an additional change with implications on renal function during sepsis.

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## 1. Introduction

Sepsis represents a major health-care burden. In the United States, the annual case incidence for severe sepsis exceeds 750,000 with a mortality as high as 30% [1]. Among septic patients, there is over a 40% incidence of concurrent acute kidney injury (AKI), and sepsis itself is responsible for nearly a third of all cases of AKI [2]. AKI increases the risk of death 6 to 8-fold [3] and serves as an independent risk factor for the development of chronic and ultimately end-stage renal disease with the need for dialysis dependence and the inherent associated resource utilization, complications, and health-care expenditure [4].

* *Corresponding author*. Department of Surgery, University of Pittsburgh, F1267PUH, 200 Lothrop St, Pittsburgh, PA 15213. Tel.: +1 412 692 2850; fax: +1 412 647 1448.
E-mail address: zuckerbraunbs@upmc.edu (B. Zuckerbraun).
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Sepsis creates a state of altered oxygen metabolism, termed dysoxia. This is thought to be mediated through effects on the mitochondria, perhaps in part of a cellular adaptive response, that results in downregulation of cytochrome oxidases and state of cellular "hibernation" [5]. The response resulting in sepsis-mediated AKI is complex and multifactorial, with interplay between inflammatory signaling, microcirculatory dysfunction, and alterations in cellular bioenergetics [6]. Despite a great deal of research focused on understanding the basic mechanisms of septic AKI, treatment remains largely supportive with hope of renal recovery. There are no current effective targeted interventions to prevent sepsis-associated AKI.

The purpose of this study was to investigate specific metabolic and biochemical changes in kidneys in response to sepsis elucidated in a murine model of cecal ligation and puncture (CLP) through the use of metabolomics. This process allows for the rapid interpretation of a wide array of biomolecules to assess for global changes in the renal metabolic profile.

## 2. Methods

### 2.1. Cecal ligation and perforation

The University of Pittsburgh Institutional Animal Care and Use Committee approved the animal protocols. Experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals. CLP was performed on C57BL/6 male mice (Jackson Laboratories, Bar Harbor, ME) aged 6–8 wk and weighing 20–25 g. These animals were anesthetized with pentobarbital (70 mg/kg, intraperitoneal). A 1–2-cm midline laparotomy was performed, and the cecum was identified. The stool was then manipulated to the tip of the cecum, which was subsequently ligated 1 cm from the tip with a 2–0 silk tie. The cecum was then double-perforated with a 22 gauge needle and returned into the abdomen. The muscle and skin were closed with a running 2–0 silk suture. Sham-operated animals underwent laparotomy and bowel manipulation without ligation or perforation. Eight mice were included in each experimental group. Tissue collection occurred at 8 h after CLP. No antibiotics were used. Animals had free access to food and water both preoperatively and postoperatively.

### 2.2. Measurements of organ injury

Blood samples were obtained from cardiac puncture at 8 h after CLP. Cystatin C was determined from serum using a mouse cystatin C kit according to manufacturer's instructions (R&D Systems, Minneapolis, MN). Serum concentrations of blood urea nitrogen and creatinine (Cr) were determined with a Heska Dri-Chem 4000 Chemistry Analyzer (Loveland, CO).

### 2.3. Biochemical profiles

Global biochemical profiles were performed on kidneys of C57BL/6 mice harvested 8 h after CLP. Tissue was sent to Metabolon (Metabolon Inc, Durham, NC) for analysis using gas chromatography/mass spectrometry or liquid chromatography/tandem mass spectrometry. Classification assays were used to illustrate reproducibility of results. A random forest assay was used. This makes multiple decision trees from a random known sample from either the CLP or sham-operated group. These decision trees are used to apply other unknown samples to the correct group. A Welch's two-sample *t*-test was calculated to identify significance between experimental groups. Significance was defined at $P \leq 0.05$.

## 3. Results

Experimental sepsis results in kidney injury with increases in serum cystatin C, blood urea nitrogen, and Cr in the CLP group as compared with the sham group at 8 h (Table 1).

Experimental sepsis results in changes to macromolecular metabolism in the kidney. Values for 329 metabolites were obtained. The overall predictive accuracy of classification was 100% as determined by random forest assay. This highly suggests a varied metabolic profile in the kidney in response to CLP. Of the 329 biochemicals analyzed, 99 were significantly different ($P < 0.05$) between treatment groups, with 63 elevated and the remaining 36 diminished in CLP compared to controls.

There was increase in glucose in the kidneys in the setting of CLP, with a concurrent increase in glycolytic intermediates (2- and 3-phosphoglycerate, pyruvate). Levels of sorbitol were elevated in CLP indicating shunting of excess glucose through the sorbitol pathway. Acetyl-CoA was undetectable in both groups. There was no significant difference in lactate levels between treatments (Fig. 1). Flux through the tricarboxylic acid (TCA) cycle appears to be reduced as evident by decreased intermediates of the pathway in the setting of CLP (Fig. 2).

Inflammatory markers were elevated in response to CLP including corticosterone, methionine sulfoxide, and polyunsaturated fatty acid–derived metabolites (12-HETE). Concurrently, there were decreased levels of antioxidants including ascorbate, a-tocopherol, and ergothioneine. These findings are consistent with increased oxidative stress induced by CLP (Table 2).

Levels of osmotic regulators varied in CLP. There was a significant increase in pinitol, urea, and taurine levels. A significant decrease was noted in betaine. There were no significant changes in glycerophosphocholine or sorbitol between groups (Table 3).

**Table 1 – Mean serum measurements for markers of renal function in sham ($n = 8$) and CLP ($n = 8$) at 8 h.**

| Test of renal function/injury | Sham | CLP |
|---|---|---|
| Cystatin C | 25 ± 5.8 ng/mL | 107 ± 21.2 ng/mL |
| Blood urea nitrogen | 19.4 ± 7.5 mg/dL | 51 ± 10.3 mg/dL |
| Creatinine | 0.17 ± 0.03 mg/dL | 0.42 ± 0.1 mg/dL |