Optical methods for the assessment of microvascular perfusion and oxygenation
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MULTI-LEVEL PHOSPHORIMETRY FOR STUDYING MICROVASCULAR AND INTERSTITIAL OXYGEN TENSIONS IN THE RENAL CORTEX AND MEDULLA DURING ENDOTOXEMIA

Adapted from

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Shock (in press)
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ABSTRACT

The pathophysiology of sepsis-induced acute kidney injury remains poorly understood. As changes in renal perfusion and oxygenation have been shown, we aimed to study the short-term effects of endotoxemia on microvascular and interstitial oxygenation in the cortex and medulla, in conjunction with global and renal hemodynamics. To this end, in a 4-hour rat model of endotoxemia, we simultaneously assessed renal artery blood flow and microvascular and interstitial oxygen tensions (µPO₂ and tPO₂, respectively) in the renal cortex and medulla using ultrasonic flowmetry, dual wavelength phosphorimetry, and tissue oxygen tension monitoring, respectively. We found that medullary microvascular and interstitial oxygen tensions decreased promptly in line with macrovascular blood flow and that changes in cortical oxygenation were only seen later on. During the entire experimental protocol, the gradient between µPO₂ and tPO₂ remained unchanged in both cortex and outer medulla. At study end, urine output was significantly decreased despite a maintained oxygen consumption rate. Hence, in this 4-hour rat model of endotoxemia, total renal oxygen consumption and the gradient between µPO₂ and tPO₂ remained unaltered, despite falls in renal perfusion and oxygen delivery and urine output.
INTRODUCTION

During endotoxemia, the balance between renal microcirculatory oxygen delivery and cellular oxygen consumption is significantly disturbed. Although oxygen diffusion, which relies on radial oxygen gradients between the microvasculature and tissue, represents a crucial step in cellular oxygen delivery, no studies have simultaneously assessed renal microvascular and interstitial oxygen tensions under endotoxemic conditions. The kidney is a high metabolic rate organ with a complex microvascular structure that is particularly prone to injury from sepsis and other inflammatory insults [Uchino et al., 2005]. Although early recognition of renal hypoperfusion and prompt resuscitation ameliorates the degree of renal dysfunction, the lack of significant progress in its prevention and management can be attributed to a still incomplete insight into the underlying pathophysiological mechanisms. Of note, glomerular filtration rate is often reduced in resuscitated animals, despite restored systemic hemodynamics and renal blood flow [Thijs and Thijs, 1998].

We previously reported that short-term endotoxemia had a variable effect on tissue oxygenation in different organ systems [Dyson et al., 2007]. While bladder tissue oxygenation rose and liver tissue oxygenation fell, there was no change in resting skeletal muscle or renal cortex. We also showed that renal microvascular oxygenation is highly sensitive to endotoxemia [Johannes et al., 2006; 2009a]. These and other conflicting results [Linder et al., 1974; Gullichsen, 1991; James et al., 1996], leave the role of oxygen (or lack thereof) in endotoxemia-induced acute renal failure uncertain.

Consequently, we aimed to study the complex changes associated with endotoxemia that occur in different compartments of the kidney (cortex and medulla) and at different levels of cellular oxygen delivery (microvasculature and interstitium) in relation to global and renal hemodynamics. To this end, we simultaneously assessed renal artery blood flow and microvascular and interstitial oxygen tensions in the renal cortex and medulla using ultrasonic flowmetry, dual wavelength phosphorimetry, and tissue oxygen tension monitoring, respectively, in a 4-hour endotoxic rat model. We hypothesized there would be a discrepancy between the effects of endotoxemia at the microvascular and interstitial levels, which would be reflected by changed oxygen tension gradients.

MATERIALS AND METHODS

In vitro comparison

An in vitro comparison was made between oxygen tensions measured by a custom-made dual-wavelength phosphorimeter (Academic Medical Center, Amsterdam, The Netherlands) and large area sensor [LAS™] probes connected to an Oxylite™ monitoring system (Oxford Optronix, Oxford, UK). This was performed at normal
physiological pH (7.4) and temperature (37 °C) in an open flask containing water with 2% bovine serum albumin (Sigma-Aldrich, St Louis, MO) and 10 μM Oxyphor G2 (Oxygen Enterprises, Philadelphia, PA). The oxygen tension was decreased from 160 (ambient) to 0 mmHg in continuous fashion by blending pure N₂ and O₂. Twenty five measurements were made within this range.

**In vivo study**

Male Wistar rats (Harlan, Horst, The Netherlands) of approximately 300 g body weight were used in all experiments. Prior to use, animals were housed in cages of six on an alternating 12 hour light–dark cycle with free access to food and water. All experiments were reviewed and approved by the Animal Research Committee, Academic Medical Center, University of Amsterdam. Care and handling of the animals was in accordance with the guidelines of the Institutional Animal Care and Use Committees (IACUC). Anesthesia was induced by intraperitoneal (i.p.) administration of sodium pentobarbital (60 mg/kg; ASTfarma, Oudewater, The Netherlands). The abdomen, chest and neck were depilated and sufentanil (20 μg/kg; Janssen-Cilag, Tilburg, The Netherlands) was administered i.p. prior to surgery. Animals were placed on a heated mat to maintain core body temperature at 37°C, assessed using a rectal thermometer inserted.

A 3 cm vertical incision was made in the neck with the salivary glands separated by careful blunt dissection. A tracheostomy was rapidly sited using 2.08 mm external diameter polythene tubing (Dräger Medical Inc, Telford, PA), and connected to a neonatal ventilator (Dräger Medical Inc), adjusted to maintain end-tidal PCO₂ at 35-40 mmHg. The left common carotid artery and right internal jugular vein were located, isolated and cannulated using 0.96 mm outside diameter PVC tubing (Biocorp Ltd, Huntingdale, Australia). Both vascular lines were flushed with 0.1 ml heparinized saline (Baxter Healthcare, Utrecht, The Netherlands) and a small quantity of blood drawn back to ensure correct placement and patency of the lines. The arterial line was connected to a pressure transducer and anesthesia subsequently maintained by continuous i.v. infusion of propofol (10 μg/kg/h; Fresenius Kabi, Utrecht, The Netherlands) and sufentanil (7.5 μg/kg/h). A continuous i.v. fluid infusion of Ringer’s lactate solution (Baxter Healthcare, Utrecht, The Netherlands) was administered at a rate of 20 ml/kg/h for the duration of the experiment to ensure adequate filling throughout (determined from pilot studies).

The bladder was cannulated through a midline laparotomy using 1.57 mm external diameter polythene tubing (Portex Ltd, Hythe, UK) inserted through a small incision at the apex. To allow access to the renal vasculature, the cecum and small intestine were wrapped in cling film and placed outside the abdominal cavity. The left kidney was carefully isolated from surrounding fatty tissue, decapsulated and immobilized in a Lucite kidney cup (K. Effenberger, Pfaffingen, Germany). A perivascular ultrasonic flow probe (1 mm diameter; Transonic Systems, Ithaca,
was coated in a water-soluble lubricant and placed around the left renal artery to measure renal macrovascular blood flow. This probe has been validated for use in anesthetized small animal models [Welch et al., 1995]. Blood flow is measured using two acoustic signals; the difference between the upstream and downstream transit times reflect the rate of blood flow [Tabrizchi et al., 2000].

Following instrumentation, 6 mg/kg Oxyphor G2 dissolved in Ringer’s lactate was administered at a rate of 20 ml/kg/h over ten minutes with the background fluid infusion temporarily suspended. Animals were allowed to settle for 30 minutes to achieve stable baseline variables (Figure 1b), and then randomly divided into two groups; sham-operated controls (n=5) and endotoxemia (n=6). Endotoxemia was induced by intravenous infusion of 2.5 mg/kg endotoxin (E.coli lipopolysaccharide, serotype 0127:B8, Sigma, Zwijndrecht, The Netherlands) given over 15 minutes. Both groups were monitored for a further four hours prior to sacrifice. An additional bolus of Oxyphor G2 (2 mg/kg in 0.3 ml Ringer’s lactate) was administered halfway through each experiment (i.e., at 120 min, Figure 1b).

Figure 1. Surgical set-up (a) and experimental protocol (b). Pre-experiment Oxyphor G2 infusion; 6 mg/kg, mid-experiment Oxyphor G2 bolus; 2 mg/kg. Endotoxin was infused over a 15 minute period.
**Microvascular oxygen tension**

Two fiber-optic light guides encased in a common bundle of 5.5 mm diameter were fixed 1 mm above the surface of the kidney and connected to a custom-made phosphorimeter for measurement of microvascular \( \text{PO}_2 \) (\( \mu \text{PO}_2 \)) [Johannes et al., 2006]. The phosphor compound used in this study (Oxyphor G2; Oxygen Enterprises, Philadelphia, PA) readily binds to macromolecules such as albumin, thus ensuring oxygen measured by the phosphorimetry technique is confined to the microvascular compartment. Using two distinct excitatory wavelengths of light allows the measurement of microvascular oxygen tensions within the kidney at two depths. Using anatomically reconstructed kidney slices, we have previously shown penetration to depths of 0.5 and 4 mm with 440 and 632 nm light wavelengths, respectively. These depths correspond to the renal cortex and outer medulla. The principles of dual-wavelength phosphorimetry are fundamentally identical to those for the tissue oxygen sensors described below; lifetimes of emitted light (around 800 nm) are inversely proportional to the local microvascular \( \text{PO}_2 \), according to the Stern-Volmer equation: 

\[
\frac{t_0}{t_1} = 1 + (k_q t_0 \times [\text{O}_2]),
\]

where \( t_0 \) is the decay time at zero oxygen, \( t_1 \) is the decay time at a specific oxygen concentration \([\text{O}_2]\) and \( k_q \) is a quenching rate constant that denotes the probability of a photoluminescent molecule and oxygen molecule colliding [Stern and Volmer, 1919]. As luminescence decay is longer at a lower \( \text{PO}_2 \), accurate measurements can be made within the physiologic range (0-80 mmHg).

**Tissue oxygen tension**

Large area sensor (LAS™) oxygen optodes (0.7 mm in diameter) connected to an Oxylite™ tissue monitoring system were inserted into the left kidney to depths of 1-2 mm and 3-4 mm, and later withdrawn to 0-1 mm and 2-3 mm to prevent anomalous measurements resulting from local hematoma. This allowed continuous tissue oxygen tension (tPO2) monitoring in the renal cortex and outer medulla, respectively [Whitehouse et al., 2006]. The sensor works by sending pulses of light (475 nm) along a fiber-optic cable to a platinum-complex photoluminophore situated at the tip of the probe. Upon excitation, the luminophore emits light (600 nm) back to the detection unit, the lifetime of which is inversely proportional to the local \( \text{PO}_2 \), according to the Stern-Volmer equation [Stern and Volmer, 1919].

**Data analysis**

Mean arterial pressure, macrovascular renal blood flow and microvascular oxygen tensions were continuously recorded using custom-made software programmed in LabView 6.1 (National Instruments, Austin, TX). Tissue oxygen tensions were recorded at 15 minute intervals. Urine output was measured in the last 30 minutes of each experiment. At the end of each experiment, carotid arterial and renal venous hemoglobin, oxyhemoglobin saturation and \( \text{PO}_2 \) were measured using
a blood gas analyzer (ABL-505 Analyzer, Radiometer, Copenhagen, Denmark) allowing a number of oxygen-derived parameters to be calculated. Arterial oxygen content was calculated as $\text{CaO}_2$ [ml/l]=$(\text{Hb} \times \text{SaO}_2 \times 1.39) + (\text{PaO}_2 \times 0.003)$. Renal venous oxygen content was calculated as $\text{CrvO}_2$ [ml/l]=$(\text{Hb} \times \text{SrVO}_2 \times 1.39) + (\text{PrvO}_2 \times 0.003)$. Renal oxygen delivery was calculated as $\text{DO}_{2\,\text{ren}}$ [ml/min]=$\text{CaO}_2 \times \text{RBF}$. Renal oxygen consumption was calculated as $\text{VO}_{2\,\text{ren}}$ [ml/min]=$(\text{CaO}_2 - \text{CrvO}_2) \times \text{RBF}$. Renal oxygen extraction ratio was calculated as $\text{O}_2\text{ER\%} = \frac{(\text{CrvO}_2 - \text{CaO}_2) \times 100}{\text{CaO}_2}$. Here, RBF is renal blood flow [ml/min], Hb is the hemoglobin level [g/dl], PaO$_2$ is the arterial partial pressure of oxygen [mmHg], PrvO$_2$ is the renal venous partial pressure of oxygen [mmHg], SaO$_2$ is the arterial hemoglobin saturation [%], SrvO$_2$ is the renal venous hemoglobin saturation [%].

All raw and calculated data are expressed as median [range] and analyzed using Prism 5.0 (GraphPad Software, San Diego, CA). In vitro calibration data were analyzed using linear regression analysis and Bland-Altman tests. Temporal hemodynamic and renal oxygenation measurements were analyzed using the Friedman test (non-parametric, repeated measures) followed by Dunn’s test for post-hoc comparisons. All other data were analyzed using the non-parametric Mann-Whitney test.

**RESULTS**

**In vitro comparison**

A tight correlation was found between the Oxylite™ and phosphorimeter-derived oxygen measurements ($r^2=0.99$; $p<0.001$; plot not shown). Bland-Altman testing also showed the two methods to be comparable (bias of -6.6 mmHg with limits of agreement of -15.8 and 2.6 mmHg; plot not shown). As photoluminescence/phosphorescence decay is shorter at high PO$_2$ values, oxygen measurements became less accurate. However, a bias of -6.6 mmHg corresponds to <10% over the range 0-80 mmHg.

**In vivo study**

All animals survived for the duration of the experiment (240 minutes). All hemodynamic and renal oxygenation measurements in sham-operated control animals remained constant throughout the experiment ($p>0.05$ compared to baseline; not shown). Induction of endotoxemia caused the characteristic biphasic drop in blood pressure and renal blood flow with an immediate decrease in blood pressure (from 95 [77–121] mmHg at baseline to 46 [22–66] mmHg at 15 minutes) followed by partial recovery within an hour. The second drop in blood pressure was more protracted but similar in magnitude to the first (34 [33–51] mmHg at 240 minutes). A similar pattern was observed for renal artery blood flow (Figure 2). Oxygen tensions were higher in the microvasculature in both anatomical compartments measured (Figure 2). Oxygen measurements
were also higher in the renal cortex compared to the outer medulla. Differences were seen in the cortical and medullary \( \text{PO}_2 \) responses to endotoxin. Whereas oxygen tensions in the outer medulla were affected by the immediate drop in blood pressure and renal blood flow, cortical oxygen tensions remained stable. In line with improvements in blood pressure and renal blood flow, medullary oxygenation recovered to baseline level within 30 min. During the later phase of the experiment, medullary and cortical oxygenation both decreased in line with the secondary decline in blood pressure and renal blood flow. After three hours, cortical and medullary oxygenation were both significantly decreased.

**Figure 2.** Effects of endotoxemia on global and renal hemodynamics and renal oxygenation. *\( p < 0.05 \) vs baseline. \( \mu \text{PO}_2 \); microvascular oxygen tension, \( t \text{PO}_2 \); tissue oxygen tension.
with respect to baseline. During the entire experimental protocol, the gradient between µPO\(_2\) and tPO\(_2\) remained unchanged in both the cortex and the outer medulla (Figure 2).

At study end (4 hours), carotid arterial and renal venous blood gas analyses were performed, allowing a number of oxygen-derived parameters to be calculated (Figure 3, Table 1). Arterial PO\(_2\), hemoglobin levels, saturation, and oxygen content were similar in sham-operated and LPS-treated animals. By contrast, a reduction in renal venous PO\(_2\), oxyhemoglobin saturation, and oxygen content was observed, albeit not significantly (p=0.06; Table 1). Whereas calculated renal oxygen delivery decreased by 65% (3.6 [2.6–6.2] ml/min vs 1.1 [0.5–2.5] ml/min for sham-operated and endotoxin-treated animals, respectively; p<0.05), renal oxygen consumption was similar in sham- (0.6 [0.3–1.1] ml/min) and endotoxin-treated (0.5 [0.3–0.6] ml/min) animals (p=0.41; Figure 3). Oxygen extraction was significantly higher (48 [25–62]% vs 16 [5–29]%) in endotoxemic animals (Figure 3), thus oxygen consumption was maintained despite the concurrent decrease in renal oxygen delivery. Urine output in endotoxin-treated animals was significantly depressed in endotoxin-treated animals (500 [360–740] ml/min vs 80 [0–470] ml/min; p<0.01; Figure 3). Endotoxemic animals also became acidemic with higher arterial base deficit values at study end compared with sham-operated controls (p<0.05; Table 1).

**Figure 3.** Effects of endotoxemia on renal oxygen-derived variables and urine output at the end of each experiment. *p<0.05 vs sham.
In the present study we aimed to investigate the complex changes associated with endotoxemia that occur in different compartments of the kidney (cortex and medulla) and at different levels of cellular oxygen delivery (microvasculature and interstitium) in relation to global and renal hemodynamics. To this end, we simultaneously assessed renal artery blood flow and microvascular and interstitial oxygen tensions in the renal cortex and medulla using ultrasonic flowmetry, dual wavelength phosphorimetry, and tissue oxygen tension monitoring, respectively, in a 4-hour endotoxic rat model. We found that in the first, immediate phase of endotoxemia, renal medullary oxygen tensions decreased and then recovered in line with renal blood flow, whereas cortical oxygen tensions remained stable. By contrast, in a second phase occurring after a few hours, both medullary and cortical oxygenation decreased in line with renal blood flow. Even though renal blood flow and oxygen delivery were markedly decreased and urine output fell, overall renal oxygen consumption was maintained as was the gradient between microvascular and interstitial PO2 in both cortex and outer medulla.

While we have previously shown in a well-fluid resuscitated model that short-term (3-hour) endotoxemia did not affect renal cortical tissue oxygenation [Dyson et al., 2007], microvascular oxygenation in this region was highly sensitive to endotoxemia [Johannes et al., 2006, 2009a]. Hence, we hypothesized that there would be a discrepancy between the effects of endotoxemia at the microvascular

### Table 1. Effects of endotoxemia on arterial and renal venous blood gas parameters and oxygen derived-variables.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Endotoxin</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial oxygenation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin [g/dl]</td>
<td>15.0 (14.3 – 16.0)</td>
<td>15.9 (14.6 – 18.2)</td>
<td>0.33</td>
</tr>
<tr>
<td>PaO2 [mmHg]</td>
<td>257 (188 – 280)</td>
<td>251 (191 – 258)</td>
<td>0.56</td>
</tr>
<tr>
<td>SaO2 [%]</td>
<td>96 (94 – 98)</td>
<td>96 (94 – 97)</td>
<td>0.90</td>
</tr>
<tr>
<td>CaO2 [ml/l]</td>
<td>194 (185 – 203)</td>
<td>205 (189 – 231)</td>
<td>0.39</td>
</tr>
<tr>
<td><strong>Renal venous oxygenation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PrvO2 [mmHg]</td>
<td>63 (40 – 97)</td>
<td>32 (10 – 55)</td>
<td>0.06</td>
</tr>
<tr>
<td>SrvO2 [%]</td>
<td>79 (68 – 91)</td>
<td>50 (32 – 55)</td>
<td>0.06</td>
</tr>
<tr>
<td>CrvO2 [ml/l]</td>
<td>161 (141 – 175)</td>
<td>100 (78 – 158)</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial base deficit [mmol/l]</td>
<td>4.1 (1 – 5.2)</td>
<td>10.6 (7.7 – 16.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>Arterial Na+ [mmol/l]</td>
<td>140 (137 – 147)</td>
<td>140 (129 – 144)</td>
<td>0.54</td>
</tr>
<tr>
<td>Arterial K+ [mmol/l]</td>
<td>4.0 (3.7 – 5.0)</td>
<td>5.8 (4.9 – 7.6)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

CaO2; arterial oxygen content, CrvO2; renal venous oxygen content, PaO2; arterial partial pressure of oxygen, PrvO2; renal venous partial pressure of oxygen, SaO2; arterial hemoglobin saturation, SrvO2; renal venous hemoglobin saturation.

### DISCUSSION AND CONCLUSIONS

In the present study we aimed to investigate the complex changes associated with endotoxemia that occur in different compartments of the kidney (cortex and medulla) and at different levels of cellular oxygen delivery (microvasculature and interstitium) in relation to global and renal hemodynamics. To this end, we simultaneously assessed renal artery blood flow and microvascular and interstitial oxygen tensions in the renal cortex and medulla using ultrasonic flowmetry, dual wavelength phosphorimetry, and tissue oxygen tension monitoring, respectively, in a 4-hour endotoxic rat model. We found that in the first, immediate phase of endotoxemia, renal medullary oxygen tensions decreased and then recovered in line with renal blood flow, whereas cortical oxygen tensions remained stable. By contrast, in a second phase occurring after a few hours, both medullary and cortical oxygenation decreased in line with renal blood flow. Even though renal blood flow and oxygen delivery were markedly decreased and urine output fell, overall renal oxygen consumption was maintained as was the gradient between microvascular and interstitial PO2 in both cortex and outer medulla.

While we have previously shown in a well-fluid resuscitated model that short-term (3-hour) endotoxemia did not affect renal cortical tissue oxygenation [Dyson et al., 2007], microvascular oxygenation in this region was highly sensitive to endotoxemia [Johannes et al., 2006, 2009a]. Hence, we hypothesized that there would be a discrepancy between the effects of endotoxemia at the microvascular
level compared to those at the interstitial level, which would be reflected by changed oxygen tension gradients. However, in contrast, we found that the gradient between µPO$_2$ and tPO$_2$ remained unchanged in both cortex and outer medulla, indicating that the microvascular and interstitial oxygen tensions remained matched and that renal oxygen consumption rate could be maintained in both renal compartments by increasing oxygen extraction, at least in the short-term. Hence, in our model, renal oxygen uptake was not limited by the diffusion driving pressure as the gradient between microvascular and interstitial PO$_2$ was maintained, even under conditions of depressed renal oxygen delivery and potential edema and inflammation associated with endotoxemia.

The maintained level of renal oxygen consumption is in agreement with that found in another rodent endotoxic study [Heemskerk et al., 1997]. By contrast, oxygen consumption during hemorrhage decreased in line with oxygen delivery, thereby maintaining tissue oxygen tension [Schlichtig et al., 1991]. Although, activated neutrophils consume oxygen to generate free radicals, the inflammatory cell infiltrate into the kidney is not large and thus unlikely to explain the maintained level of oxygen consumption. Heemskerk et al. postulated that the proximal tubular epithelium may become more leaky as a result of vascular inflammation which, theoretically, could result in more back-flux of sodium and an increased workload required for reabsorption [Heemskerk et al., 1997]. Furthermore, Weber et al. demonstrated that the quantity of sodium reabsorbed to oxygen consumed decreases during endotoxemia, thus implying increased energy expenditure for tubular transport and/or renal synthetic activities [Weber et al., 1992]. Taken in conjunction with the decrease in urine output and a worsening in plasma biochemistry, our results could represent either a functional renal impairment or, arguably, an adaptive response [Thurau and Boylan, 1976]. By decreasing glomerular filtration rate in response to a diminished renal blood flow, less energy would need to be expended by the tubules to reabsorb large volumes of salt and water, especially in the presence of increased vascular leak, thereby protecting the kidney from irreversible or, at least, long lasting damage. The oliguria could thus be viewed as a protective response to prevent uncontrolled urine loss due to a failure to reabsorb sufficient fluid.

It could be alternatively hypothesized that a functional renal impairment (falling urine) in combination with sufficient microvascular oxygenation (i.e., a non-compromised oxygen tension gradient between microvasculature and interstitium) could be related to occult hypoxic areas that are not detected by the applied techniques [Johannes et al., 2009a,b,c; Bezemer et al., 2010c]. Previously, phosphorescence lifetime deconvolution, a method allowing the retrieval of the PO$_2$ histograms that make up the average PO$_2$ value as measured in the present study, revealed that the acute drop in mean medullary µPO$_2$ following endotoxin administration was due to a homogenously low µPO$_2$ [Johannes et al., 2006, 2009a]. Conversely, the cortical µPO$_2$ was highly heterogeneous with a large
fraction of low μPO₂ values that did not exist prior to endotoxemia. This increase in cortical heterogeneity however did not overly affect the average μPO₂ value, as also reflected in the current study during the early phase of endotoxemia.

Interpretation of the results presented here should be done with great care as several methodological considerations exist. First, Bland-Altman analysis revealed a small bias between the Oxyphor- and Oxylite-mediated PO₂ measurements; i.e., Oxyphor-mediated (microvascular) measurements were slightly, but consistently, 7 mmHg higher than the Oxylite-mediated (interstitial) measurements. Second, the dual-wavelength phosphorimeter used in this study measures a mixture of arteriolar, capillary, and venular microcirculatory oxygen tensions. While longitudinal gradients between each of these vessel types clearly exist, a consistently higher PO₂ was observed (significantly higher than the measurement bias revealed by Bland-Altman analysis) in the microvasculature compared to that in the interstitium. Third, a matter of potential concern when using albumin-bound Oxyphor for microvascular oxygen tension measurements is for albumin leakage into the extravascular space, especially under endotoxemic conditions. If the albumin-bound Oxyphor would leak into the extravascular space, the gradient between the measured Oxyphor-derived ‘microvascular’ oxygen tension and the Oxylite-derived interstitial oxygen tension should become smaller over time. In the lower two panels of Figure 2 the gradient does not significantly differ in both the sham and endotoxin groups. Hence, this indicates that while some Oxyphor did leak into the extravascular space, this did not significantly affect our measurements and conclusions (at least for the time span of the present study). Fourth, There may also be issues with potential artefacts caused by local damage related to optode insertion. However, the responsiveness and consistent direction of the tissue oxygen measurements to changes in the macrocirculation in this and other studies [Dyson et al., 2007, 2009] do suggest reliability. The large surface area (up to 8 mm² in tissue contact) of the optodes used in this study provide an averaged result within the tissue bed rather than a ‘pinpoint’ reading that may be proximal or distal to a nutrient vessel.

Furthermore, this study has some limitations. Bolus administration of endotoxin to a rat model causes an overwhelming innate immune response that has similarities, though many disparities, to human and large-mammal sepsis [Langenberg et al., 2006, 2007; Chvojka et al., 2008]. Secondly, our findings are only applicable to this short-term rat model of acute endotoxemia associated with an initial marked hypotension and hypoperfusion. We did not address changes in flow and oxygen supply-demand balance that may occur later in the course of endotoxemia. The present study does not allow delineation between those effects directly related to hemodynamic alterations and those related to a direct inflammatory response. Next, the microvascular anatomy of the rat significantly differs from that of higher order species. Thus, observed distributive effects of endotoxemia on renal oxygenation may differ in humans and should not be directly translated to clinical scenarios. For example, the elongated medulla
of rat kidneys may have a different tolerance to ischemia than the medulla of human kidneys. Finally, in the present study, the renal microcirculation is broadly divided into two compartments: medulla and cortical. The renal microcirculation, however, is actually far more complex and the methodology applied does not allow us focus upon specific functional anatomical structures (e.g., glomerular, peritubular, outer versus inner medulla).

In conclusion, by simultaneous assessment of renal artery blood flow and microvascular and interstitial oxygen tensions in the renal cortex and medulla in a 4-hour rat model of endotoxemia, we found distinctive temporal changes in the different regions. While the gradient between \( \mu \text{PO}_2 \) and \( t \text{PO}_2 \) was higher than that reported in metabolically less active organs, this did not significantly change during a short-term endotoxemic insult. Despite the maintained oxygen consumption (reflected by both the oxygen tension gradient between the microvasculature and the interstitium and direct computation from blood gas analysis and renal blood flow), urine output was significantly decreased. This study provides integrative insight of the complex changes associated with endotoxemia that occur in different compartments of the kidney (i.e., cortex and medulla) and at different levels of cellular oxygen delivery (i.e., microvasculature and interstitium) in relation to global and renal hemodynamics.