Tissue oxygen monitoring in rodent models of shock

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Abstract

Tissue oxygen tension (tPO₂) reflects the balance between local oxygen supply and demand and could thus be a useful monitoring modality. However, both the consistency and amplitude of the tPO₂ response in different organs during varied cardiorespiratory insults is unknown. We therefore investigated the effects of endotoxemia, hemorrhage and hypoxemia on tPO₂ measured in deep and peripheral organ beds. Using an anesthetised Wistar rat model, we monitored arterial pressure (MAP), blood gas and lactate levels, descending aortic and renal blood flow, and tPO₂ in skeletal muscle, bladder epithelium, liver and renal cortex during (i) a 10 mg/kg lipopolysaccharide infusion, (ii) sequential removal of 10% of circulating blood volume and (iii) reductions in inspired oxygen concentration. Comparison was made against sham-operated animals. Different patterns were seen in each of the shock states with condition-specific variations in the degree of acidemia, lactatemia, and tissue oxygen responses between organs. Endotoxemia resulted in a rise in bladder tPO₂, an early fall in liver tPO₂ but no significant change in muscle and renal cortical tPO₂. Progressive hemorrhage however produced proportional falls in liver, muscle and bladder tPO₂ but renal cortical tPO₂ was maintained until profound blood loss had occurred. By contrast, progressive hypoxemia showed proportional falls in tPO₂ in all organ beds. This study highlights the heterogeneity of responses in different organ beds during varied shock states that are likely related to local changes in oxygen supply and utilization. Whole body monitoring is not generally reflective of these changes.
Key words: Tissue oxygenation, tissue oxygen tension, hemodynamics, rat, endotoxemia, hemorrhage, hypoxemia, shock.
Introduction

Tissue oxygen tension (tPO$_2$) represents the balance between local supply and demand of oxygen. Under resting conditions, the level will vary both between and within organs, being higher in tissues with low metabolic rates such as the bladder (35; 36), and lower in more metabolically active tissues such as brain (20) liver (48), gut (45), renal cortex and medulla (51).

Decreases in tPO$_2$ have been recorded across numerous organ beds during low oxygen transport states such as hemorrhage, hypoxemia and heart failure. This is implicit of an inability of the regional oxygen supply to match continuing metabolic demands. These beds range from conjunctiva (18; 42), subcutaneous tissue (15), bladder (44) and muscle (6; 28) to deeper organs such as liver (33; 48) and kidney (51). The fall from baseline levels generally corresponds to the severity of the insult (27; 44). By contrast, sepsis, the systemic inflammatory response to infection, produces an elevated tPO$_2$. This suggests either mitochondrial dysfunction and/or a metabolic shutdown with a consequent reduction in oxygen utilization despite local availability. This phenomenon has been witnessed in organs as diverse as bladder and skeletal muscle in both septic patients (4; 30; 38) and animal models (35; 36). Furthermore, a rise in tPO$_2$ is still seen in low cardiac output sepsis provided adequate fluid resuscitation has been given (35; 36).

Measurement of tPO$_2$ clearly offers a potentially useful diagnostic/monitoring tool for the critically ill patient in whom current monitoring techniques are
generally global, non-specific and often insensitive. However, only a small number of studies have simultaneously assessed tPO$_2$ in more than one organ bed (28; 33; 45), thus inter-organ differences are largely unknown. This is particularly pertinent when considering the use of accessible peripheral tissues such as bladder and muscle as a surrogate for monitoring changes in deeper, more vital organs such as liver and kidney. Accordingly, we sought to determine hemodynamic and tissue oxygen responses to endotoxemia, hemorrhage and hypoxemia in a short-term, anesthetised rat model, comparing peripheral (muscle and bladder) and deep (liver and renal cortex) organ beds.
Methods

Male Wistar rats of approximately 300 g body weight were used in all experiments. Prior to instrumentation, animals were housed in cages of six on a light–dark cycle of 12–12 h with free access to food and water. All experiments were performed according to Home Office (United Kingdom) guidelines under the 1986 Scientific Procedures Act.

Spontaneously breathing animals, anesthetised by 5% isoflurane in room air, were placed onto a heated mat to maintain rectal temperature at 36.5-37.5 °C. Under 2% isoflurane, the left common carotid artery and right internal jugular vein were cannulated using 0.96 mm outside diameter PVC tubing catheter (Biocorp Ltd, Huntingdale, Australia). The arterial line was connected to a pressure transducer (Powerlab, AD Instruments, Chalgrove, UK) for continuous monitoring of mean arterial pressure. A tracheostomy was sited using 2.08 mm external diameter polythene tubing (Portex Ltd, Hythe, UK) to secure and suction the airway; this was connected to a T-piece to maintain anesthesia. 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. Anesthesia was then reduced to 1.2% for the remainder of the experiment. To allow access to the abdominal vasculature, the cecum and small intestine were wrapped in cling film and placed outside the abdominal cavity. The left renal artery and descending aorta were isolated from surrounding tissue by careful blunt dissection. Ultrasonic flow probes (Transonics Systems, Ithaca, NY, USA) of 1 and 2 mm
diameter were coated in a water-soluble lubricant and placed around the left renal artery and descending aorta, respectively to measure blood flow. Insertion of large area surface (LAS™) oxygen sensors (0.7 mm in diameter) connected to the Oxylite™ tissue monitoring system (Oxford Optronix, Oxford, UK) allowed continuous monitoring of tPO2 in muscle, bladder, liver and renal cortex. The LAS™ sensor is a new, large-area, sensing device in which the oxygenation measurement takes places along the shaft of the catheter, i.e. away from the tip and site of maximal tissue trauma. The relatively large sensing area of the LAS™ sensor averages spatial micro-environmental tPO2 fluctuations, thereby making it less sensitive to tissue movement and placement. The sensor sends short pulses of light (475 nm) along a fiber-optic cable to a platinum-complex fluorophore situated 2 mm from the tip of the probe, and provides a total measurement tPO2 surface area of 8 mm² in contact with the tissue. Upon interaction with oxygen, the fluorophore emits light (600 nm) back to the detection unit, the lifetime of which is inversely proportional to the local PO2 within the tissue of interest. As the fluorescence decay is longer at a lower PO2, accurate measurements can be made within the physiologic range (0-60 mmHg). Unlike polarographic techniques, oxygen is not consumed during the measurement process.

For muscle tPO2, a small incision was made at mid-thigh level, and the sensor inserted into the vastus intermedius muscle to a depth of 10 mm using an 18-gauge guidance cannula. The left kidney was punctured using a 22-gauge needle and the oxygen probe inserted to a depth of 2 mm before later being withdrawn by 1 mm to prevent anomalous measurements resulting from local
hematoma. An insertion depth of 1 mm enables measurement of tPO₂ within the renal cortex as previously described (51). The bladder oxygen probe was sited within the bladder lumen via the bladder catheter which continually drained the bladder, thus ensuring good sensor contact with the epithelial surface. Sampling liver tPO₂ was achieved by placing the probe directly into the airtight space between two of the liver lobes. Pilot studies showed comparable data to that obtained from direct puncture though without inducing any trauma.

Mean arterial pressure, blood flow in left renal artery and descending aorta and all tPO₂ measurements were continuously monitored and recorded onto a computer using a 16-channel Powerlab system and Chart 4.2 acquisition software (AD Instruments, Chalgrove, UK).

Following instrumentation, intravascular volume optimization was achieved by repeated 1.5 ml intravenous fluid challenges given over 10 seconds every 5 minutes until blood pressure or aortic blood flow failed to increase >10%. This ensured adequate filling at baseline. A continuous fluid infusion of normal saline was then administered at a rate of 20 ml/kg/h for the duration of the experiment to ensure adequate filling throughout. Cling film was placed over the abdomen to minimise evaporative fluid and convective heat losses. Rectal temperature was recorded at baseline and at 15 minute intervals for the duration of the experiment using a TES 1319 thermometer (TES Electrical Electronic Corp, Taipei, Taiwan) inserted to a depth of 3 cm.
Animals were allowed to stabilize for at least 30 minutes to achieve stable baseline physiologic variables. Sham-operated controls were then monitored for a further three hours prior to sacrifice. Arterial blood samples (approximately 0.2 ml) were taken into heparinised capillary tubes for blood gas analysis (ABL-70 analyser, Radiometer, Copenhagen, Denmark) at prespecified timepoints stated below. This analysis included measurement of arterial base excess, lactate and \( \text{PO}_2 \). In separate studies, a variety of cardiorespiratory insults were administered: (i) endotoxemia was induced by intravenous infusion of 10 mg/kg endotoxin (\text{E.coli} lipopolysaccharide, serotype 0127:B8, Sigma, Poole, UK) over 5 minutes; (ii) controlled hemorrhage was achieved by removal of 10\% of estimated circulating blood volume (based on a total of 70 ml/kg) from the arterial line into a heparinised syringe which was then kept at 37 °C on a heated mat. This was re-infused into the animal 15 minutes later to observe the effects of re-transfusion. Every 15 minutes thereafter, 10\% of circulating blood volume was removed until death occurred; (iii) hypoxemia was achieved by varying the percentage of inspired oxygen (\( \text{FiO}_2 \)) by blending pure oxygen and nitrogen through a flowmeter via the isoflurane vaporiser while still maintaining anesthesia. The oxygen content of the gas mixture was assessed prior to delivery by passing a sample through the blood gas analyser. 15\% \( \text{O}_2 \) was delivered for 30 minutes followed by normoxia for 30 minutes, then 12.5\% \( \text{O}_2 \) for 30 minutes, a further 30 minute period of normoxia and, for the final hour or until death occurred, 10\% \( \text{O}_2 \).
In control and endotoxin-treated animals, arterial blood gas analysis was performed at baseline (0 h), 0.5, 1 and 3 hours. In hemorrhagic shock, analysis took place at baseline and at 30 minute intervals thereafter using the exsanguinated blood. In hypoxemic rats, blood gas analysis was performed at baseline and at the end of each period of altered FiO₂ (i.e. at 30 minute intervals). As some animals subjected to consecutive hemorrhage or hypoxemia died before the 3 h timepoint point, efforts were made to take a pre-terminal (PT) sample.

Data are presented as mean ± standard error (n=6-10 per group). Statistics were performed on raw data using a repeated measures two-way ANOVA followed by Tukey’s post-hoc test (SigmaStat, Systat Software Inc, San Jose, CA, USA) to compare multiple groups at multiple time points.
**Results**

Prior to instrumentation, the bodyweight of each study group of rats was similar, averaging 305.6 ± 4.3 g. All animals in the control (n=10) and endotoxin-treated (n=9) groups survived for the 3 h duration of the experiment. All animals (n=6) subjected to sequential hemorrhage died before 3 h, with the average (± SE) time of death being 127.3 ± 10.8 minutes. In the hypoxemia group, four out of six animals died before 3 h (average time to death 149.8 ± 10.2 minutes).

At baseline, i.e. after volume optimization and a stabilization period, none of the groups showed statistically significant differences from one another for any cardiorespiratory variable measured. Baseline values of tPO2 were highest in the bladder, followed by muscle, liver and renal cortex (Figs 1-3).

All measurements in control animals remained constant for the duration of the experiment with the exception of aortic blood flow (ABF) and bladder tPO2 which progressively fell, and kidney cortical tPO2 which gradually rose (p<0.05) (Figures 1-3). Core temperature in the hemorrhage and hypoxemia groups did not differ from time-matched controls at any point during the experiment. Treatment with endotoxin significantly increased core temperature to a maximum of 39.5 ± 0.3 °C at 3 h compared with time-matched controls (37.5 ± 0.3 °C, p<0.001). Arterial CO2 (PaCO2) remained constant in control animals throughout the experiment (38.7 ± 2.4 mmHg baseline, 31.7 ± 2.6 mmHg at 3 h, p=0.1).
The effects of endotoxin on hemodynamic, tissue oxygen and arterial blood gas variables are shown in Figure 1. The predictable early fall in blood pressure, aortic and renal blood flow was seen, followed by recovery within an hour. In this model, flows subsequently fell (p<0.05 compared to control) but blood pressure remained unchanged and hemoglobin was elevated compared with baseline (p<0.01, Figure 4). This suggests that normovolemia was not maintained, likely due to increased capillary leak despite the high volume resuscitation being given concurrently. Calculated oxygen delivery was decreased at 3 h compared with baseline (p<0.05, Figure 4). Arterial lactate and base excess became progressively deranged (p<0.001 compared to control), with significance achieved as early as 1 h post-injection. Arterial PO\textsubscript{2} and percentage saturation (HbO\textsubscript{2}) remained unchanged in both control and LPS-treated rats while different patterns were observed in the tissue oxygen levels in the four organ beds studied. Whereas bladder tPO\textsubscript{2} increased over controls from as early as 1 h (overall ANOVA p<0.01), renal cortical tPO\textsubscript{2} (p=0.68) and muscle tPO\textsubscript{2} (p=0.14) showed no significant change, while liver tPO\textsubscript{2} fell significantly (p<0.05) from 2 h onwards. Arterial PO\textsubscript{2} remained constant throughout (76.5 ± 6.5 mmHg baseline, 73.4 ± 9.8 at 3 h, p=0.8) whereas PaCO\textsubscript{2} decreased (34.8 ± 1.6 mmHg baseline, 21.5 ± 3.4 at 3 h, p<0.01).

Figure 2 shows the effects of sequential hemorrhage. Initial removal of 10% blood volume only significantly affected mean arterial pressure, which fell from 92.7 ± 2.5 to 78.2 ± 1.6 mmHg at 15 minutes (p<0.05). This recovered upon
re-infusion of the shed blood. Subsequent exsanguination caused a progressive decrease in mean arterial pressure with pre-terminal values averaging 44.2 ± 4.9 mmHg (p<0.001). Similar changes were seen in aortic and renal blood flow (p<0.01). Arterial base excess (ABE) and lactate were maintained within normal limits until 40% of blood volume had been removed, at which point rapid and progressive deterioration was noted for both variables (p<0.01, Figures 2 and 6). Arterial PO$_2$ remained constant for 1 h before increasing to a maximum of 107.9 ± 6.9 mmHg from a baseline value of 80.9 ± 2.4 mmHg (p<0.001). This rise in arterial PO$_2$ is related to hypocapnia secondary to metabolic acidosis-induced hyperventilation. Accordingly, arterial PCO$_2$ levels decreased from 39.6 ± 1.5 mmHg at baseline to pre-terminal values of 14.5 ± 1.6 mmHg (p<0.001). Tissue oxygen tensions in muscle, bladder and liver were all reduced by a similar proportion following progressive hemorrhage (p<0.001 compared to controls), and fell in line with oxygen delivery (Figure 5). In contrast, renal cortical tPO$_2$ rose at the 1 h timepoint (p<0.05), implying decreased utilization in excess of the reduction in supply. Upon further exsanguination, the renal cortical tPO$_2$ only fell below control values at the pre-terminal phase (p<0.05). Blood hemoglobin content also decreased (11.1 ± 0.5 g/dL baseline, 6.7 ± 0.2 at 2 h, p<0.001) whereas arterial oxygen saturation elevated with the increased respiratory effort (89.7 ± 1.1 % baseline, 95.6 ± 0.5 % at 2 h, p<0.001, Figure 4). Oxygen delivery progressively decreased over time (5.6 ± 1.0 l/min baseline, 1.0 ± 0.2 at 3 h, p<0.001, Figure 4).
The effects of hypoxemia on the measured cardiorespiratory variables are illustrated in Figure 3. Increasing the level of hypoxemia resulted in falls in all variables, albeit at different timepoints. Blood pressure and tissue PO₂ values were affected early (while breathing 15% O₂), blood flows and oxygen delivery (Figure 4) fell at a later stage (on 12.5% O₂), whereas arterial base excess only decreased at the pre-terminal phase, while breathing 10% O₂. Arterial PO₂ initially fell in line with the degree of hypoxemia but rose at 10% O₂ due to hyperventilation-induced hypocapnia related to the profound metabolic acidosis. Similarly, the oxyhemoglobin level initially fell in line with the FiO₂ but remained unchanged at 10% O₂ (Figure 4). Arterial CO₂ was reduced during hypoxemia albeit not significantly different to baseline (36.6 ± 0.8 mmHg baseline, 23.4 ± 9.3 mmHg pre-terminal, p<0.1). During the periods of normoxemia, tissue PO₂ returned to control values in all beds (except the bladder after 12.5% O₂), whereas blood pressure and blood flows showed no evidence of recovery. Of note, arterial lactate showed only a non-significant (p=0.12) rise, even when there was marked acidemia (Figures 3 and 6).

The relationship between global oxygen delivery (DO₂) and tPO₂ in the different organs is illustrated in Figure 5. Despite the different baseline values, the proportionality in terms of fall in tPO₂ was often maintained across the organ beds. Muscle tPO₂ showed the most consistency across the different insults followed by the liver, although the fall in hepatic tPO₂ occurring sooner with endotoxin. Renal cortical and bladder tPO₂ showed less consistency.
Discussion

Tissue oxygen tension represents the balance between local oxygen supply and demand. It has been previously used in clinical studies of heart failure, sepsis, blood loss and the adequacy of resuscitation from a variety of sites including conjunctiva (42), subcutaneous tissue (18) and skeletal muscle (4). However, the relevance of changes in these more peripheral beds with regard to deeper, ‘vital’ organs such as the liver and kidney has not been previously addressed. We set out to answer this question by constructing models of three pathophysiologically distinct shock states (hemorrhage, hypoxemia and early sepsis) that share common features of severe cardiorespiratory and biochemical derangement.

We achieved reasonable hemodynamic stability in our sham-operated model over the time course of the experiment. In terms of macro-cardiorespiratory variables, only aortic blood flow fell from baseline. However, hematocrit was maintained, suggesting maintenance of intravascular filling, and there was no significant change in oxygen delivery. Renal cortical tPO2 increased over the course of the experiment despite no change in macrovascular renal blood flow. As renal blood flow predominantly supplies the cortex, this implies an impairment of cortical oxygen utilisation and/or a relative decrease in microvascular shunting as renal venous pO2 is usually very high (49). This could be related to surgical stress and/or trauma from direct puncture resulting in release of inflammatory mediators including nitric oxide, or to a potential metabolic effect of isoflurane anesthesia on the kidney.
Our study demonstrates heterogeneous responses in tissue oxygen tension to the different cardiorespiratory insults in the four organ beds studied. These vary both between organs during the course of the same insult, and in the same organ during different shock states. Alterations in peripheral tPO₂ (bladder, skeletal muscle) did mirror those recorded in central organs (liver, renal cortex) during hypoxemia, both in terms of direction and magnitude. In contrast, the renal cortical tPO₂ response to hemorrhage was considerably delayed in comparison to the other organ beds, whereas endotoxemia resulted in a much wider variation, ranging from an early rise in bladder tPO₂ to an early fall in hepatic tPO₂.

Our results challenge the traditional paradigm that a fall in oxygen delivery by whatever means, i.e. a reduction in cardiac output, hemoglobin or oxygen saturation, will produce a similar response at the organ level. They also highlight increased sensitivity and/or adaptation of individual organs to specific insults. For example, we have previously argued that sepsis-induced organ failure represents a state of metabolic shutdown consequent to impaired mitochondrial energy generation (7; 8). This line of thought was initiated by our finding of an elevated bladder epithelial tPO₂ in both high and low cardiac output short-term endotoxemic rat models (35; 36), indicative of a reduced oxygen demand relative to supply. Similar findings were reported in gut mucosa (46) and human skeletal muscle (4; 38). As >90% of total body oxygen consumption is directed towards mitochondrial respiration, an
elevated tPO$_2$ is highly suggestive of decreased mitochondrial utilization, and/or a metabolic shutdown with a consequent reduction in oxygen demand.

In the present study we replicated our earlier finding of a rise in bladder epithelial tPO$_2$ during short-term endotoxemic sepsis (35; 36). However, this did not translate to either muscle or renal cortex, where no significant change in tPO$_2$ was noted from control values despite a fall in aortic and renal blood flow, nor in the liver where an early and marked fall was seen. This is likely to reflect early specific organ responses to endotoxin, ranging from a metabolic shutdown in the bladder to an increase in hepatic oxygen demand. Notably, Fry et al (17) reported a decline of nearly 90% in hepatic tissue PO$_2$ in a 6-hour endotoxemic rat model, despite these animals maintaining normal systemic oxygenation and blood pressure. Ince has described a ‘microcirculatory and mitochondrial distress syndrome’ where microcirculatory and mitochondrial dysfunction persist despite apparent restoration of the macrocirculation (21). Our results support this concept though individual responses are seen in different organ beds, at least in this short-term model.

There are several reasons that could potentially explain an early fall in liver tPO$_2$. Firstly, Dahn et al (12) reported increases in hepatic blood flow (72%) and splanchnic oxygen consumption (60%) in septic patients, yet suggested from modelling data that intrahepatic flow redistribution would significantly reduce centrilobular oxygen tension. Secondly, microcirculatory heterogeneity may lead to increases in local hypoxic regions, and consequently could limit oxygen availability in septic (5; 14; 21; 39) as well as
hemorrhagic (50) shock states. Thirdly, hepatic metabolic activity is augmented during early sepsis as part of the acute phase response (11), and this may continue despite a fall in oxygen delivery. Activation of Kupffer cells, which constitute 15% of liver mass, would greatly increase oxygen consumption due to both hypermetabolism and free radical generation within these macrophages. This may form a considerable component of the increased liver oxygen utilization seen during sepsis. Fourthly, portal venous oxygen saturation may be considerably lower due to decreased gut flow and/or any increase in gut metabolic activity. In a recent short-term septic dog study, Rahal et al (34) reported a decrease in portal venous oxygen saturation from 91.7 ± 1.4% to 59.8 ± 6.1% at 150 minutes in conjunction with a fall in cardiac output. This is particularly pertinent as the portal vein contributes 75% of hepatic blood flow (25).

It is important to stress the severe, short-term (3 h) nature of the endotoxin challenge we applied. This was designed to match the time courses of the other lethal insults studied, but it will engender a different physiologic response to a more protracted septic insult. In a mouse model of endotoxic sepsis, liver tPO₂ also decreased by approximately 75% at 6 hrs (25). Notably, liver oxygen extraction fell concurrently from 41% in control animals to 15% following endotoxin, implying a significant fall in utilization over and above the decrease in tissue oxygenation. In a separate study, the same group reported recovery of liver tPO₂ towards normal values if the animals survived the 6-hour timepoint (26). Using a 15-hour murine septic shock model, Albuszies et al reported impaired hepatic metabolic capacity despite
well-maintained hepatosplanchnic oxygenation and microvascular perfusion (1) while MacMicking et al reported oxidative damage to the liver only after 10-11 hours following an endotoxin challenge (31). This may be related to the high antioxidant capacity of the liver, in particular glutathione, which protects mitochondria against oxidant and nitrosative damage (9). As a consequence, effects of sepsis on mitochondrial activity in the liver may be delayed with respect to other organs. Kozlov and colleagues found a decrease in mitochondrial respiratory activity in cardiomyocytes but a concurrent rise in liver mitochondria (29). We have previously demonstrated mitochondrial dysfunction in both liver and muscle in a long-term (72 h) awake, fluid-resuscitated rat model of fecal peritonitis (8). Only muscle ATP/ADP ratios were significantly reduced at the 4-hour timepoint, whereas liver mitochondrial function was more affected than muscle at 24 and 48 hours. In forthcoming experiments of prolonged sepsis we plan to study temporal changes in tissue oxygen tension in different beds in order to better understand individual organ responses.

In contrast to endotoxemia, we observed decreased oxygen tensions for liver, muscle and bladder in both hemorrhagic shock and hypoxemia. These observations are consistent with a fall in oxygen delivery related to decreases in cardiac output and arterial oxygen content. Decreases in tissue oxygenation following hemorrhage have been reported by other groups in both peripheral tissues such as skeletal muscle (6) and conjunctiva, (6; 18; 42) and deeper tissues such as liver and gut (28; 33; 48).
Notably, the renal cortical tPO2 response to hemorrhage did not follow that seen in other organ beds until the pre-terminal stage. This may be related to regional flow autoregulation that, as we and others have previously shown (32; 51), is better maintained in the cortex than in the medulla during hemorrhage. Unlike other organ beds, renal oxygen consumption varies directly according to oxygen delivery, both in the normal physiological range and during hemorrhagic shock, as shown by Schlichtig and colleagues (41). This is in contrast to the liver where the same group found metabolic demands exceeded oxygen delivery during hemorrhage (40). This could therefore explain both the maintained renal cortical tissue oxygen tension and the early decrease in hepatic tPO2 we observed both during hemorrhage (and endotoxemia). Tubular sodium reabsorption is traditionally considered to account for 70-90% of renal oxygen consumption (41). As renal oxygen consumption is delivery-dependent, a fall in oxygen supply should result in decreased sodium and water reabsorption, and thus polyuria. This clearly does not happen in shock states thus the reduction in oxygen consumption must be secondary to reduced glomerular filtration.

There was a four-fold variation in baseline tPO2 in the four organ beds studied. Comparable data have been cited by ourselves (35; 43; 44; 51) and others (2; 23; 37; 48) and reflects differences in their individual metabolic activity and oxygen supply. The platinum optode used in this study calculates oxygen tension over a relatively large area using the principle of fluorescence quenching. This is related directly to the local tPO2 according to the Stern-Volmer equation. As the fluorescence lifetime is longest at low tPO2 values,
these probes are most sensitive in the physiological range of 0–60 mmHg. The probe also samples from a relatively large volume of tissue compared with many other probes and may thus be less susceptible to small variations in probe position in proximity to blood vessels.

Consistent with our previous findings in this spontaneously breathing, anesthetised rat model (35; 36), the arterial PO$_2$ increases and PaCO$_2$ falls (data not shown) as a result of compensatory hyperventilation for severe metabolic acidosis. This hyperventilation could even increase arterial PO$_2$ at 10% inspired O$_2$ concentration above that measured at 12.5% inspired O$_2$. However, this was not reflected in tissue oxygen tension in any of the organ beds monitored as blood flow was also significantly reduced with lower inspired oxygen concentrations.

Acute derangements in arterial base excess and lactate are used clinically as indicators of poor organ perfusion. These indices correlate well with severity of illness and mortality (3; 13; 16). Though hyperlactatemia is traditionally considered a marker of tissue hypoxia, it is now clear that it can be elevated by non-anaerobic mechanisms in critical illness. This includes increased activity of the Na$^+$/K$^+$-ATPase ion pump in skeletal muscle related to increased catecholamine levels (19; 22) and accelerated glycolysis (24). Other causes include an inability to metabolise pyruvate to acetyl CoA, due to decreased activity of pyruvate dehydrogenase (47), and a decrease in mitochondrial respiration that may be due to direct inhibition (3; 13; 16) or substrate (oxygen) limitation (24). Figure 6 shows the relationship between
arterial base deficit and lactate. Accordingly, hemorrhage produced equivalent increases in blood levels of both variables although, with pre-terminal hypoxemia, there was considerable deterioration in the base deficit level with little change in lactate. With endotoxin, the blood lactate and base deficit levels were equivalent to that seen in hemorrhage and mild-moderate hypoxemia. We did not measure blood pyruvate levels, nor blood cations and anions to draw comparisons between the different shock states in terms of calculated anion gap and strong ion difference. Precise mechanisms remain to be determined to explain the disparity during late hypoxemia, although one component may be increased consumption of lactate by the liver, heart and other organs as a fuel substrate. Alternatively, hypoxia has been shown to inhibit the Na⁺/K⁺-ATPase ion pump (10) which could account for the absence of hyperlactatemia coupled with a severe metabolic acidosis in late hypoxemia.

In summary, we demonstrate heterogenous responses in tissue oxygen tensions in different organ beds to a variety of short-term cardiorespiratory insults. Only hypoxemia produced comparable, severity-dependent changes across all organs. The renal cortex appears to initially protect itself by decreasing its oxygen consumption during hemorrhage, in contrast to the liver which is more compromised than the other organs studied during endotoxemia. The response in arterial lactate and base deficit also varied between insults and did not necessarily reflect major deterioration or protection in any individual organ. This study highlights both the utility of assessing organ hypoperfusion from either global markers such as lactate, or
from tissue oxygen tension in surrogate organ beds such as the bladder. However, it also demonstrates the limitations of these techniques in that specific organs may be differentially affected by different conditions that may not be apparent either from markers monitored globally or in other tissues.
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Legends for figures

Figure 1: Effects of endotoxemia on hemodynamic, tissue oxygen and blood gas biochemical variables. Control - white circles, Endotoxin - black squares. MAP - mean arterial pressure; ABF - aortic blood flow; RBF - renal blood flow; ABE - arterial base excess; tPO₂ - tissue oxygen tension. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey’s test, *p<0.05 comparing control to endotoxemia, †p<0.05 comparing endotoxemia timepoint to baseline, °p<0.05 comparing control timepoint to baseline.

Figure 2: Effects of hemorrhage on hemodynamic, tissue oxygen and blood gas biochemical variables. Control - white circles, Hemorrhage - black diamonds. MAP - mean arterial pressure; ABF - aortic blood flow; RBF - renal blood flow; ABE - arterial base excess; tPO₂ - tissue oxygen tension. 3 * denotes pre-terminal values for hemorrhage treatment group. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey’s test, *p<0.05 comparing control to hemorrhage, †p<0.05 comparing hemorrhage timepoint to baseline, °p<0.05 comparing control timepoint to baseline.

Figure 3: Effects of hypoxemia on hemodynamic, tissue oxygen and blood gas biochemical variables. Control - white circles, Hypoxemia - black symbols (21% FiO₂ - triangles, 15% FiO₂ - diamonds, 12.5% - FiO₂ squares, 10% FiO₂ - circles). MAP - mean arterial pressure; ABF - aortic blood flow; RBF - renal
blood flow; ABE - arterial base excess; tPO₂ - tissue oxygen tension; FiO₂ - percentage inspired oxygen. 3* denotes pre-terminal values for hypoxemia treatment group. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey’s test, *p<0.05 comparing control to hypoxemia, †p<0.05 comparing hypoxemia timepoint to baseline, ‡p<0.05 comparing control timepoint to baseline.

Figure 4: Effects of endotoxemia, hemorrhage and hypoxemia on oxygen delivery, hemoglobin and oxyhemoglobin (HbO₂). Control - white circles, Endotoxemia - black squares, Hemorrhage - dark grey diamonds, Hypoxemia - light grey triangles. Oxygen delivery was calculated as the product of descending aortic blood flow, hemoglobin, arterial oxygen saturation (HbO₂) and 1.34. 3* denotes pre-terminal values for hemorrhage and hypoxemia treatment groups. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey’s test, *p<0.05 compared to control, †p<0.05 comparing timepoint to baseline.

Figure 5: Relationship between global oxygen delivery and tissue oxygen tension in response to continued endotoxemia, progressive hemorrhage and increasing hypoxemia. Control - white circles, Endotoxemia - black squares, Hemorrhage - dark grey diamonds, Hypoxemia - light grey triangles. DO₂ - oxygen delivery, tPO₂ - tissue oxygen tension. Data represent mean from each group.
Figure 6: Comparison of continued endotoxemia, progressive hemorrhage and increasing hypoxemia on arterial base excess and lactate. Control - white circles, Endotoxemia - black squares, Hemorrhage - dark grey diamonds, Hypoxemia - light grey triangles. Data represent mean ± standard error from each group.
Figure 1: Effects of endotoxemia on hemodynamic, tissue oxygen and blood gas biochemical variables. Control - white circles, Endotoxin - black squares. MAP - mean arterial pressure; ABF - aortic blood flow; RBF - renal blood flow; ABE - arterial base excess; tPO2 - tissue oxygen tension. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey's test, *p<0.05 comparing control to endotoxemia, †p<0.05 comparing endotoxemia timepoint to baseline, op<0.05 comparing control timepoint to baseline.
Figure 2: Effects of hemorrhage on hemodynamic, tissue oxygen and blood gas biochemical variables. Control - white circles, Hemorrhage - black diamonds. MAP - mean arterial pressure; ABF - aortic blood flow; RBF - renal blood flow; ABE - arterial base excess; tPO2 - tissue oxygen tension. 3* denotes pre-terminal values for hemorrhage treatment group. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey's test, *p<0.05 comparing control to hemorrhage, †p<0.05 comparing hemorrhage timepoint to baseline, op<0.05 comparing control timepoint to baseline.
Figure 3: Effects of hypoxemia on hemodynamic, tissue oxygen and blood gas biochemical variables. Control - white circles, Hypoxemia - black symbols (21% FiO2 - triangles, 15% FiO2 - diamonds, 12.5% FiO2 - squares, 10% FiO2 - circles). MAP - mean arterial pressure; ABF - aortic blood flow; RBF - renal blood flow; ABE - arterial base excess; tPO2 - tissue oxygen tension; FiO2 - percentage inspired oxygen. 3* denotes pre-terminal values for hypoxemia treatment group. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey's test, *p<0.05 comparing control to hypoxemia, †p<0.05 comparing hypoxemia timepoint to baseline, op<0.05 comparing control timepoint to baseline.
Figure 4: Effects of endotoxemia, hemorrhage and hypoxemia on oxygen delivery, hemoglobin and oxyhemoglobin (HbO2). Control - white circles, Endotoxemia - black squares, Hemorrhage - dark grey diamonds, Hypoxemia - light grey triangles. Oxygen delivery was calculated as the product of descending aortic blood flow, hemoglobin, arterial oxygen saturation (HbO2) and 1.34. 3* denotes pre-terminal values for hemorrhage and hypoxemia treatment groups. Data expressed as mean ± standard error. Statistics on raw data: Two-way ANOVA with repeated measures followed by Tukey’s test, *p<0.05 compared to control, †p<0.05 comparing timepoint to baseline.
Figure 5: Relationship between global oxygen delivery and tissue oxygen tension in response to continued endotoxemia, progressive hemorrhage and increasing hypoxemia. Control - white circles, Endotoxemia - black squares, Hemorrhage - dark grey diamonds, Hypoxemia - light grey triangles. DO2 - oxygen delivery, tPO2 - tissue oxygen tension. Data represent mean from each group.
Figure 6: Comparison of continued endotoxemia, progressive hemorrhage and increasing hypoxemia on arterial base excess and lactate. Control - white circles, Endotoxemia - black squares, Hemorrhage - dark grey diamonds, Hypoxemia - light grey triangles. Data represent mean ± standard error from each group.