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An ovine model of hyperdynamic endotoxemia and vital organ metabolism

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ABSTRACT

Background: Animal models of endotoxemia are frequently used to understand the pathophysiology of sepsis and test new therapies. However, important differences exist between commonly used experimental models of endotoxemia and clinical sepsis. Animal models of endotoxemia frequently produce hypodynamic shock in contrast to clinical hyperdynamic shock. This difference may exaggerate the importance of hypoperfusion as a causative factor in organ dysfunction. This study sought to develop an ovine model of hyperdynamic endotoxemia and assess if there is evidence of impaired oxidative metabolism in the vital organs.

Methods: Eight sheep had microdialysis catheters implanted into the brain, heart, liver, kidney and arterial circulation. Shock was induced with a 4hr escalating dose infusion of endotoxin. After 3hrs vasopressor support was initiated with noradrenaline and vasopressin. Animals were monitored for 12hrs after endotoxemia. Blood samples were recovered for haemoglobin, white blood cell count, creatinine and proinflammatory cytokines (IL-1Beta, IL-6 & IL-8).

Results: The endotoxin infusion was successful in producing distributive shock with the mean arterial pressure decreasing from 84.5 $\pm$ 12.8mmHg to 49 $\pm$ 8.03 mmHg($p<0.001$). Cardiac index remained within the normal range decreasing from 3.33 $\pm$ 0.56 l/min/m^2 to 2.89l $\pm$ 0.36 l/min/m^2 ($p=0.0845$). Lactate/pyruvate ratios were not significantly abnormal in the heart, brain, kidney or arterial circulation. Liver microdialysis samples demonstrated persistently high lactate/pyruvate ratios (mean 37.9$\pm$3.3).

Conclusions: An escalating dose endotoxin infusion was successful in producing hyperdynamic shock. There was evidence of impaired oxidative metabolism in the liver suggesting impaired splanchnic perfusion. This may be a modifiable factor in the progression to multiple organ dysfunction and death.

Keywords: Sepsis; endotoxemia; resuscitation; microdialysis; haemodynamic monitoring

Introduction

Sepsis is a leading cause of admission to intensive care with an estimated 2 million patients annually(8). For those unfortunate enough to progress to septic shock mortality may be in excess of 50%(35).

Hypotension is one of the defining characteristics of septic shock and secondary hypoperfusion has been thought to be critical in the development of multiple organ dysfunction(26). This has been the rationale to use fluid resuscitation as the first therapeutic intervention in septic shock in order to reverse perfusion deficits and prevent progression to organ dysfunction(8, 26). Experimentally this has been supported by numerous models of endotoxemic shock(2, 33, 34) with improved outcomes when cardiac output is increased with fluid resuscitation(22, 29). While the animal literature demonstrates consistent benefit with fluid resuscitation, the clinical evidence remains equivocal. Observational studies of fluid resuscitation have suggested both benefit(11) and harm(27) with the only randomised control trial of fluid resuscitation demonstrating increased mortality(18). This discrepancy in effect may be due to differences in the type of shock between clinical sepsis and commonly used experimental models. When administered to humans, endotoxin produces the characteristic hyperdynamic response seen in clinical sepsis(31). However, in both small and large animal models of endotoxemia, endotoxin frequently produces hypodynamic shock with rapid and severe reductions in cardiac output (1, 2, 12, 22). The models used to demonstrate a benefit with fluid resuscitation are all notable for being hypodynamic models of shock (3, 21, 23, 25). It is conceivable that the therapies aimed at increasing cardiac output such as fluid resuscitation may have differing effects in hypo and hyperdynamic shock.

Similarly regional hypoperfusion and impaired oxidative metabolism have been proposed as central mechanisms in the development of organ dysfunction in septic shock(14, 26). This has been supported experimentally by a number of animal models demonstrating decreased regional blood flow across the vital organs (1, 3). Again notably these observations have been reported in hypodynamic models of sepsis and endotoxemia.

This highlights the need for models of sepsis that reflect the clinical presentation seen in humans in order to better understand commonly used resuscitative therapies. Consequently, we sought to develop an ovine model of endotoxemia capable of producing hyperdynamic shock. For the purpose of this investigation we defined hyperdynamic shock as hypotension below a mean arterial pressure of 60mmHg with normal or increased cardiac output. Additionally, as hypoperfusion and impaired oxidative metabolism are thought to be a potential contributor to the development of organ dysfunction, we aimed to describe the metabolic changes that occur in the vital organs during hyperdynamic endotoxemic shock supported with vasopressors in the absence of fluid resuscitation.

Materials and Methods

Animal preparation

The study was approved by the Queensland University of Technology (QUT) Office of Research Ethics and Integrity (QUT: 1400000032).

Eight 3 year old non-pregnant Merino ewes weighing between 37-48kg were fasted overnight for the study. Sample size for the model was determined by reviewing previous large animal models published by the authors in order to estimate a sample size that would be adequately representative(6). Animals were sourced from the Commonwealth Scientific and Industrial Research Organisation. Animals were housed at the Queensland University of Technology medical engineering research facility with free access to shade. Prior to the experimental protocol they were fed with proprietary sheep feed and lucerne with free access to water. One animal was excluded from analysis due to bleeding and resultant anaemia occurring as a result of instrumentation. At the commencement of the study anatomical landmarks and Seldinger technique was used to insert a three lumen central venous catheter (Arrow International, Reading, PA, USA) and venous sheath (Edwards Lifesciences, Irvine, CA, USA) into the left external jugular vein with both sutured in place. Anaesthesia was induced with midazolam 0.5mg/kg (Pfizer (Perth) Bentley, WA, Australia), buprenorphine 300mcg (Reckitt Benckiser Healthcare, Hull, U.K.) and alfaxalone 3mg/kg (Jurox,

Rutherford, NSW, Australia). A cuffed endotracheal tube size 8-10F (Smiths Medical International Hythe, Kent, UK) was inserted into the trachea and animals were commenced on mechanical ventilation. Tidal volumes were maintained at 10 mls/kg via a Galileo intensive care ventilator (Hamilton Medical AG, Switzerland) with a mainstream end-tidal carbon dioxide (ETCO2) detector (Marquette TRAM, GE Healthcare, Waukesha, WI, USA) with the rate adjusted to maintain an ETCO2 of 35-45 mmHg. All animals began ventilation with a FiO2 of 30% oxygen and positive end expiratory pressure of 10 cmH20 these were adjusted according to a pre-specified scale to maintain arterial saturation >94%. Anaesthesia was maintained with an infusion of alfaxalone 6 mg/kg/hr (Jurox, Rutherford, NSW, Australia), midazolam 0.25 mg/kg/hr (Pfizer (Perth) Bentley, WA, Australia), fentanyl 15 mcg/kg/hr (Hameln Pharmaceuticals, Hameln, Germany) and ketamine 10 mg/kg/hr (Troy Laboratories, Glendenning NSW, Australia). All anaesthetic and analgesic medications were titrated to maintain adequate surgical anaesthesia.

Once anaesthetised, animals were positioned in the right lateral position. A 14 French nasogastric tube (Salem Sump, Coviden, Mansfield, MA, USA) was inserted to decompress the stomach and left on free drainage throughout the duration of the study. The left facial artery was dissected and cannulated (Vygon arterial leadercath, Ecouen, France) to facilitate continuous blood pressure monitoring and blood sampling. This was coupled to continuous data monitoring system (Solar 8000, GE Healthcare, Waukesha, WI, USA) with data recorded every five seconds with custom software. A pulmonary artery catheter (Swan-Ganz CCOmbo, Edwards Lifesciences, Irvine, CA, USA) was inserted via the venous sheath (Edwards Lifesciences, Irvine, CA, USA) and connected to a Vigilance II monitor (Edwards Lifesciences, Irvine, CA, USA) in order to record continuous cardiac output (CCO), mixed venous oxygen saturation (SvO2), central venous pressure (CVP), pulmonary artery pressure (PAP), systemic vascular resistance (SVR) and core body temperature. All haemodynamic and ventilator data was automatically recorded with a data monitoring system (Solar 8000, GE Healthcare, Waukesha, WI, USA).

The right femoral artery was dissected and cannulated in order to introduce a reference microdialysis catheter (CMA 64 MD probe Kista, Sweden). A left lateral thoracotomy was performed at approximately the 7th rib space to facilitate placement of 2 myocardial microdialysis probes into the free wall of the left ventricle (CMA 63 MD probe Kista, Sweden). A left lateral and midline laparotomy was performed to enable insertion of microdialysis probes into the left renal cortex and right lobe of the liver respectively (CMA 63 MD probe Kista, Sweden). A 4mm left hemisphere cranial burr hole was created 5mm lateral to the sagittal suture and 5mm anterior to the lamboid suture to enable introduction of cerebral microdialysis probe (CMA 70 MD probe Kista, Sweden). The microdialysis probes were inserted into each organ by first inserting the introducer cannula (CMA, Kista, Sweden) through which the microdialysis catheter was fed. The cannula was then split in-situ to leave the microdialysis catheter in place in each organ.

During surgical preparation all animals received 1L of 5% dextrose (Baxter, Deerfield, Illinois, USA) at 250 mls/hr to offset losses during overnight fasting and insensible losses during surgery. Following initial re-hydration, all animals received Hartmann's solution (Baxter, Deerfield, Illinois, USA) as maintenance fluids at 2.5 mls/kg/hr for the duration of the experiment.

Normothermia was maintained throughout with a warming blanket (Hemotherm, Cincinnati Sub Zero, Cincinnati, OH, USA).

Experimental protocol

After completion of surgical instrumentation, a 60 minute interval was allowed prior to commencement of the experimental protocol for stabilisation of the animal.

The lipopolysaccharide infusion (LPS; *E. coli* serotype O55:B5, diluted to 0.1 mcg/ml) was commenced at 0.5 mcg/kg/hr for 30 minutes, then escalated to 1 mcg/kg/hr for 30 minutes, 2 mcg/kg/hr for 30 minutes, 3 mcg/kg/hr for 30 minutes and then maintained at 4 mcg/kg/hr for a further 2 hours (total LPS dose 11.25 mcg/kg). Three hours after commencement of the LPS infusion haemodynamic support was initiated (Figure 1). Noradrenaline 60 mcg/ml in 5% dextrose

(Hospira, Lake Forest, IL, USA) was commenced in order to maintain a mean arterial pressure (MAP) between 60-65 mmHg. If the noradrenaline dose reached 20 mcg/min, vasopressin (PPC, Richmond Hill, ON, Canada) was commenced at 0.8 units/hr and increased to a maximum of 1.6 units/hr if hypotension persisted. Controlled administration of noradrenaline and vasopressin was achieved using a Gemini infusion pump (Alaris medical systems San Diego, CA, USA). Animals were monitored for 12 hours after the end of the endotoxin infusion and received a total of 13 hours of haemodynamic support. At the end of the monitoring period all animals were euthanized with intravenous sodium pentobarbitone (162.5mg/kg) (Lethabarb, Virbac, Australia).

Hourly blood gases were taken throughout the experiment and analysed on site ABL800 Flex (Radiometer, Copenhagen, Denmark). Blood was taken at baseline, at the commencement of LPS, at start of haemodynamic support, end of LPS and at 1, 6 and 12 hours during the monitoring period. Blood was analysed for haemoglobin (Hb content), white blood cell count (WBC), creatinine, interleukin 1-beta (IL-1Beta), Interleukin 6 (IL-6) and Interleukin 8 (IL-8). Hb and WBC measurements were performed using the veterinary mode of the Act diff™ hematology analyzer (Beckman Coulter Australia Pty Ltd, NSW, Australia). Creatinine was measured using a COBAS Integra 400 blood chemistry analyser (Roche Diagnostics, Australia). Quality controls (Precinorm Control Clin Chem Multi 1 and 2; Roche Diagnostics, Australia) were used for this analysis. cytokines were measured using a custom sandwich enzyme linked immune assay for sheep IL-1Beta, IL-6 and IL-8 (see reference for detailed methods)(24).

Microdialysis technique

Microdialysis probes (10 mm) with a 20,000 Dalton cut-off were used in all organs. Two catheters were used in the myocardium to account for potential catheter failure with cardiac motion. All microdialysis probes were perfused with heparinised plasma-lyte 148 (Baxter, Deerfield, Illinois, USA) at a rate of 0·3 microlitres/min to maximize recovery. Samples were recovered hourly and analysed immediately on an ISCUS clinical microdialysis analyser for lactate and pyruvate

(Hammarby Fabriksväg, Stockholm, Sweden). The lactate/pyruvate (L/P) ratio was used to detect evidence of impaired oxidative metabolism in each organ. The normal L/P ratio is between 15-20 in all tissues(32), with values greater than 25 considered evidence of impaired oxidative metabolism(32).

Statistical methods

The effect of the endotoxin infusion on the haemodynamic variables were analysed using paired T-tests comparing baseline values to those obtained prior to commencement of haemodynamic support.

For the Hb, WBC, creatinine, serum cytokines and blood gas results the distributions of variables were assessed, both overall and at baseline. Normality was assessed by inspecting histograms and qnorm plots in addition to using a skewness and kurtosis test (sk test). Most variables were normally distributed at baseline except IL-1Beta. This variable was log-transformed for use in analyses.

Mixed effects regression models were specified to determine the effects of endotoxin over time on the variables of interest. The start of the endotoxin infusion was used as the reference timepoint in all regression models. Time was fitted as a categorical variable to determine the fixed effect and random effects for sheep and time within sheep were specified. After inspecting the values for lactate and base excess, the model was re-specified with time as a continuous variable.

The outcome of interest for the microdialysis samples was whether or not the L/P ratio exceeded 25 during the period when animals were in haemodynamically-supported endotoxemic shock. The L/P ratios were not normally distributed, so a log transformation was performed. The distribution by site was assessed using Chi square analysis. The change in ratio over time within each site was explored using logistic regression models while accounting for clustering within sheep. However, the limited sample size and distribution across categories of time and site meant that some models were unstable. Hence, the continuous variable was used in analysis. The distribution of L/P ratios

were skewed, so a logarithmic transformation was applied to achieve a normally distributed outcome variable for use in modelling. The change in ratio over time was examined by fitting mixed effects linear regression models, with a random effect for sheep and a fixed effect for time. Separate models were fitted for each site. STATA (version 13) statistical software package was used for analyses.

Results

The endotoxin infusion produced systemic hypotension with mean MAP decreasing from 84.5 $\pm$ 12.8 mmHg to 49 $\pm$ 8.03 mmHg; ($p<0.001$) (figure 2B & table 1). All animals had a MAP below 60mmHg after 3 hours of endotoxemia. Reduction in systemic vascular resistance (SVRI) was the dominant factor in the development of hypotension decreasing from 1931 $\pm$ 241 dynes/cm^5/m^2 to 1198 $\pm$ 176 dynes/cm^5/m^2; ($p<0.001$)(figure 2C & table 1). There was a non-significant reduction in cardiac index which decreased from 3.33 $\pm$ 0.56 l/min/m^2 to 2.89l $\pm$ 0.36 l/min/m^2 during the endotoxin infusion ($p=0.0845$) (figure 2A & table 1). The mean pulmonary artery pressure increased from 16.5 $\pm$ 2.1 mmHg to 21 $\pm$ 4.3 mmHg ($p=0.01$) (figure 2D & table 1).

Vasopressor requirements rose throughout the support period with mean rate of noradrenaline of 50.13 $\pm$ 3.42 mcg/min (figure 3A & 3B). All animals required initiation of vasopressin to maintain target MAP. During the period of haemodynamic support, mean cardiac output was preserved, and increased from 2.89 $\pm$ 0.36 l/min/m^2 to 5.0 $\pm$ 2.39 l/min/m^2.

Mean haemoglobin was 95 $\pm$ 6 g/L at baseline and decreased with surgical instrumentation to 76 $\pm$ 14.2g/L at the commencement of endotoxin ($p<0.001$) (figure 4 & table 2). By the end of the monitoring period the haemoglobin had recovered to 86 $\pm$ 10 (p 0.043) (figure 4 & table 2). Mean WBC was 7.3 $\pm$ 1.3 10^9/L at baseline and decreased to 4.9 + 1.7 10^9/L at the commencement of the endotoxin infusion ($p<0.001$) (figure 4 & table 2). Endotoxemia was associated with a further

decrease in the WBC count reaching the lowest value at the end of the endotoxin infusion at 2.05 ± 1.2 10^9/L (figure 4 & table 2).

Serum creatinine was 81.9 ± 10.2micromol/L at baseline and was unchanged at the beginning of the endotoxin infusion at 82.4 ± 12.7micromol/L (p=0.92) (figure 4 & table 2). Endotoxemia resulted in an increase in serum creatinine reaching maximal value of 201± 37.5 (micromol/L) at the end of the monitoring period (p<0.001) (figure 4 and table 2).

Surgical instrumentation did not result in a significant change in any of the measured cytokines from baseline levels (figure 5 & table 3). Endotoxemia resulted in an increase in all measured cytokines (figure 5 & table 3). IL-1Beta was 255.3 + 247.2 at beginning of the endotoxin infusion and peaked at the end of the endotoxin infusion at 922.5 + 658.2 (p<0.001) then decreased over the monitoring period to 326.5 + 290 (figure 5). IL-6 was 34.8 + 17.8 at the beginning of the endotoxin infusion and increased with endotoxemia to 611 + 243.9 (p<0.001). IL-6 peaked 1 hour after the endotoxin infusion at 740 + 148 before decreasing to 583 + 253 at the end of the monitoring period (figure 5). IL-8 was 1196.5 + at the start of the endotoxin infusion increasing to the maximal value of 27812 + 4692 at 3 hours into the endotoxin (p<0.001).

Endotoxemia resulted in both an increase in the arterial lactate and a decrease in the base excess that was sustained throughout the monitoring period (figure 6). In the mixed effect regression models lactate rose at 0.2mmol/L/hr (CI 0.1 to 0.4mmol/hr) (P<0.001) and the base excess decreased by -0.6mmol/L/hr (CI -0.9 to -0.4mmol/L/hr) (p<0.001) over the course of the experiment (figure 6).

Microdialysis results

Summary statistics for the mean log L/P ratio and mean log transformed L/P ratios by site during the period of haemodynamic support are shown in Table 4. Mean L/P ratios prior to commencement of haemodynamic support were abnormal in the brain (28.09 SE 7.04), kidney (49.72 SE 23.79) and

liver (72.17 SE 18.34). Overall, 43% of the 420 samples analysed after the initiation of vasopressor displayed ratios exceeding 25, but this varied significantly by site ($p<0.001$) with 84% of liver samples being above 25 compared to 13% of heart samples. Mean log transformed L/P ratios were not elevated in the brain, heart or kidneys. Only the liver demonstrated a significantly elevated mean log transformed L/P ratio (37.9$\pm$3.3) during the monitoring period.

Observed changes in the L/P ratio over time are shown in Figure 7. Results from mixed effects linear regression models indicated that the L/P ratio in liver samples decreased significantly ($p<0.001$) while levels in heart samples increased (p0.007) over time during the interval following initiation of vasopressor treatment. However, overall levels in the heart were significantly lower, and in the liver were significantly higher compared to femoral arterial samples.

Discussion

Large animal models of endotoxemia are frequently used to test resuscitative therapies in sepsis and support their use clinically. This is despite significant differences in the response to endotoxemia between animals and humans. In both porcine and ovine models of endotoxemia bolus endotoxin results in rapid development of hypodynamic shock with pulmonary hypertension, reduced cardiac output and hypotension(28). Similarly in murine models of endotoxemia bolus endotoxin frequently produces hypodynamic shock(17). This is in contrast to humans where bolus endotoxin results in the development of hyperdynamic shock similar to clinical sepsis(31). This difference is thought to be due to the abundance of pulmonary intravascular macrophages (PIM) present in the lungs of both sheep and pigs that release thromboxane A2 in response to endotoxin causing pulmonary hypertension and secondary right heart dysfunction(28). Similar findings of rapid hypodynamic shock have been seen in both ovine and canine models when live *E. coli* infusions have been used to induce sepsis (1, 15). The main finding of this study was we were able to produce hyperdynamic endotoxemic shock with an increasing dose endotoxin infusion without the use of fluid resuscitation. By increasing the endotoxin infusion slowly over a period of 3 hours

we were able to minimise endotoxin induced pulmonary hypertension and maintain cardiac output in the normal range. These results build on the work of Schmidhammer *et al*. who used an exponentially increasing dose endotoxin infusion to minimise pulmonary hypertension in a porcine model of endotoxin induced acute respiratory distress syndrome(28). They hypothesised that by increasing the endotoxin dose slowly they were able to slowly deplete the PIMs of thromboxane during the endotoxin infusion, minimising the rise in MPAP. Our infusion protocol did not completely prevent a decrease in cardiac output and rise in MPAP with peak effects occurring at approximately 60 minutes into the endotoxin infusion. However, unlike other animal models of endotoxemia (1, 2, 20, 22, 30, 33) cardiac index recovered to near baseline and was within the normal range prior to commencement of haemodynamic support. Calzavacca*et al*., Langenberg *et al*. and Giantomasso *et al*. have all used bolus *E. coli* in a number of ovine models to induce hyperdynamic sepsis (4, 9, 10, 16). In each study bolus *E. coli* was successful in producing both an increase in cardiac output and reduction in MAP occurring between 6-12 hours. Notably however in all models while there was a decrease in MAP it remained above thresholds typically used when initiating resuscitative therapies (MAP 60-65mmHg) (4, 9, 10, 16). While these are excellent models of hyperdynamic sepsis they do not represent severe hyperdynamic shock and thus are not ideal for testing therapies aimed at resuscitation. Our model has the advantage of reliably producing severe systemic hypotension over a relatively short period of time (3 hours) with a MAP well below recommended treatment thresholds (49 + 8.03mmHg) making it well suited for testing acute resuscitative therapies.

Both human sepsis and experimental endotoxemia have both been shown to be associated with increases in proinflammatory cytokines such as IL-1Beta and IL-6(5, 7, 19). The levels of which have found to be predictive of both disease severity and mortality (5, 19). The results of the serum cytokine analysis confirm that our method of endotoxemia is effective in producing increases in plasma cytokines also elevated in human sepsis and models of endotoxemia. In this study both IL-1Beta and IL-8 levels peaked towards the end of the endotoxin infusion before declining to near

baseline levels over the course of the monitoring period. IL-6 levels rose throughout the endotoxin infusion and remained elevated during the monitoring period. Similarly, Calzavacca *et al*. measured serum IL-6 levels in sheep with hyperdynamic sepsis induced with *E. coli* infusion and found persistent elevation of IL-6 for 24 hours after induction of sepsis. Of note the IL-6 levels in their study rose by approximately 350% in response to bacteraemia in comparison to more than 2000% in our study. This may be due to the use of endotoxemia as the initiating insult as it is known to produce an exaggerated cytokine response when compared to models of bacterial sepsis.

With respect to the microdialysis results during the period of supported endotoxemic shock, our findings suggest that the metabolic response is not uniform across the vital organs. After induction of shock and prior to initiation of haemodynamic support, L/P ratios were abnormal in the brain and markedly abnormal in the kidney and liver. This suggests that during untreated endotoxemic shock with significant hypotension, impaired oxidative metabolism does occur in these organs. Interestingly, restoring blood pressure with vasopressors was associated with normalisation of L/P ratios in the brain and kidneys. Additionally, during the 13 hours of haemodynamic support the L/P ratios were not significantly elevated in the heart, brain or kidneys. This is in contrast to the liver, where L/P ratios were markedly abnormal at the initiation of support and remained so throughout the experiment. These results suggest that while there does not appear to be significantly impaired oxidative metabolism in the heart, brain or kidneys during supported shock, there may be on-going occult splanchnic hypoperfusion that persists despite maintenance of blood pressure targets.

Endotoxemia resulted in a consistent rise in serum creatinine over the monitoring period. This occurred while the mean kidney L/P ratio was not elevated and remained relatively stable over the same period. There are a number of possible explanations for this interesting finding. It may be that the renal dysfunction observed may not be related to inadequate perfusion and may occur through non-perfusion dependant means. Similar observations have been found by Langenberg *et al*. in an ovine model of sepsis induced by *E. coli* infusion. In their 48 hour model of sepsis there was a

dramatic increase in renal blood flow over the study, despite this creatinine rose and renal function deteriorated(16). They hypothesised that the renal dysfunction was not a result of inadequate perfusion, but rather a perfusion independent phenomenon(16). Alternatively this finding may have occurred due to the impaired oxidative metabolism that was not detected by the microdialysis catheters. The catheters were inserted into the renal cortex and are only reflective of the metabolic changes occurring around the membrane. It is possible significant hypoperfusion occurred in the renal medulla that was missed by the microdialysis catheters and accounted for the observed renal dysfunction.

This model was successful in producing severe distributive shock with noradrenaline and vasopressin doses increasing throughout the support period. One might expect a parallel deterioration in splanchnic perfusion with increasing vasopressor use in the absence of fluid resuscitation. Interestingly, while hepatic L/P ratios were abnormal there was significant improvement in the ratios over the course of the study despite the increasing noradrenaline and vasopressin use. This suggests that changes in macrocirculatory support requirements do not reliably reflect changes occurring at the microcirculatory level of individual tissues.

Relationship to previous studies

Most of the experimental literature examining regional blood flow and metabolism in sepsis and endotoxemia is in hypodynamic models of shock (1, 3, 22, 23, 25, 33, 34). These hypodynamic models have been used to demonstrate both reduced renal and splanchnic perfusion(1). Furthermore, these models support the use of fluid resuscitation to reverse these perfusion changes and transition from hypodynamic to hyperdynamic shock (13, 15). It is unclear however, if hypoperfusion and impaired metabolism occur in spontaneous hyperdynamic shock without fluid resuscitation that is seen in humans challenged with endotoxin(31).

The question of regional perfusion and metabolism in hyperdynamic sepsis has been examined by Giantomasso *et al*. who developed an ovine model of hyperdynamic sepsis with an intravenous

bolus of *E. coil*(10). Utilising continuous vascular flow probes Giantomasso *et al*. were able to demonstrate preserved or increased blood flow in the brain, heart, kidneys and splanchnic circulation in hyperdynamic sepsis. Importantly however, the animals did not develop severe shock, maintaining mean arterial blood pressures above 65 mmHg throughout the study(10). Despite increased blood flow the authors note that both myocardial dysfunction and renal dysfunction occurred. They hypothesize that this is likely due to non-ischemic injury and not the result of hypoperfusion. Our results complement their conclusions as while global blood flow was increased in their study, they were unable to exclude perfusion abnormalities at the microcirculatory level that may have accounted for the development of organ failure. Our findings support their hypothesis that organ dysfunction is not related to perfusion abnormalities in the both heart and kidneys as oxidative metabolism appeared unimpaired at the tissue level in our study. With respect to splanchnic circulation, a possible explanation for the difference between their findings of increased blood flow and the observed impaired oxidative metabolism seen in our study is the haemodynamic differences between the models. In their model animals did not require haemodynamic support and maintained blood pressures above 65mmHg throughout the study. Conversely all animals in our study had severe shock requiring high dose vasopressor therapy. It is possible that impaired splanchnic perfusion is a late feature of septic shock and only occurs in conjunction with severe hypotension as was seen in our model. Additionally, as we did not measure blood flow directly it is possible that global splanchnic blood flow was preserved and the impairments seen were the result of microcirculatory dysfunction.

Our model addresses the unanswered question of whether impaired oxidative metabolism occurs in hyperdynamic shock needing haemodynamic support in the absence of volume resuscitation. This question is of significant importance as tissue hypoperfusion is the central justification for the prioritisation of fluid resuscitation as the first line therapy in sepsis resuscitation.

Strengths and limitations

Our study has several strengths. We have developed a novel means of inducing endotoxemic shock that reliably produces hypotension with preserved cardiac output. We believe this better reflects the clinical manifestations of human septic shock, and is a more reflective model of sepsis than hypodynamic models of endotoxemia.

The use of microdialysis to assess the metabolic state of the tissues has both significant advantages and disadvantages. The ability to directly interrogate the redox state of the tissue arguably provides a more clinically relevant measure of adequacy of oxygen delivery than measures of global organ blood flow. However, measures are specific to the area directly surrounding the catheter and may miss intra-organ variability. As the renal catheters were inserted into the renal cortex, as mentioned it is possible that clinically relevant impaired metabolism was present in the renal medulla that was not detected in this study. Similarly, the use of the liver as a surrogate for splanchnic perfusion is imperfect, being supplied from both the systemic and portal circulation. This would have meant that if the L/P ratios in the liver were unaffected, we would not have been able to exclude impaired metabolism occurring in the splanchnic circulation.

Conclusion

In summary, this model was able to avoid the occurrence of cardiac dysfunction and hypodynamic shock commonly associated with endotoxemia. This resulted in a haemodynamic profile similar to that seen in human sepsis and may serve as a more representative model for future studies of resuscitative therapies. Additionally lactate/pyruvate ratios remained abnormal in the liver despite maintenance of blood pressure with vasopressors. This may reflect occult splanchnic hypoperfusion and be a potentially modifiable phenomenon in the prevention of multiple organ dysfunction and death. We can now use this model to assess if additional therapies such as fluid resuscitation or novel haemodynamic agents are effective in modifying the severity of shock and improving splanchnic metabolism.

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References

1. Bellomo R, Kellum JA, and Pinsky MR. Transvisceral lactate fluxes during early endotoxemia. *CHEST* 110: 198-204, 1996.

2. Brackett D, Schaefer C, Tompkins P, Fagraeus L, Peters L, and Wilson M. Evaluation of cardiac output, total peripheral vascular resistance, and plasma concentrations of vasopressin in the conscious, unrestrained rat during endotoxemia. *Circ shock* 17: 273-284, 1984.

3. Bressack MA, Morton N, and Hortop J. Group B streptococcal sepsis in the piglet: effects of fluid therapy on venous return, organ edema, and organ blood flow. *Circ res*61: 659-669, 1987.

4. Calzavacca P, Lankadeva YR, Bailey SR, Bailey M, Bellomo R, and May CN. Effects of selective β1-adrenoceptor blockade on cardiovascular and renal function and circulating cytokines in ovine hyperdynamic sepsis. *Crit Care* 18: 610, 2014.

5. Cannon JG, Tompkins RG, Gelfand JA, Michie HR, Stanford GG, Van Der Meer JW, Endres S, Lonnemann G, Corsetti J, and Chernow B. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 161: 79-84, 1990.

6. Chemonges S, Shekar K, Tung J-P, Dunster KR, Diab S, Platts D, Watts RP, Gregory SD, Foley S, and Simonova G. Optimal management of the critically ill: anaesthesia, monitoring, data capture, and point-of-care technological practices in ovine models of critical care. *BioMed res int*2014: 2014.

7. Copeland S, Warren HS, Lowry SF, Calvano SE, Remick D, Inflammation, and Investigators tHRtI. Acute inflammatory response to endotoxin in mice and humans. *Clin diagn lab immunol*12: 60-67, 2005.

8. Daniels R. Surviving the first hours in sepsis: getting the basics right (an intensivist's perspective). *J antimicrob chemother*66: ii11-ii23, 2011.

9. Di Giantomasso D, May CN, and Bellomo R. Norepinephrine and vital organ blood flow during experimental hyperdynamic sepsis. *Intensive care med*29: 1774-1781, 2003.

10. Di Giantomasso D, May CN, and Bellomo R. Vital organ blood flow during hyperdynamic sepsis. *CHEST* 124: 1053-1059, 2003.

11. Garland A, Kumar A, and Waechter J. EARLY ADMINISTRATION OF CRYSTALLOID FLUIDS REDUCES MORTALITY IN SEPTIC SHOCK. *Am J Respir Crit Care Med* 181: A4097, 2010.

12. Halmagyi D, Starzecki B, and Horner G. Mechanism and pharmacology of endotoxin shock in sheep. *J App Physiol*18: 544-552, 1963.

13. Hiltebrand LB, Krejci V, Banic A, and Sigurdsson GH. Redistribution of microcirculatory blood flow within the intestinal wall during sepsis and general anesthesia. *Anesthesiology*98: 658-669, 2003.

14. Hollenberg SM, Ahrens TS, Annane D, Astiz ME, Chalfin DB, Dasta JF, Heard SO, Martin C, Napolitano LM, and Susla GM. Practice parameters for hemodynamic support of sepsis in adult patients: 2004 update. *Crit care med*32: 1928-1948, 2004.

15. Lagoa CE, de Figueiredo LF, Cruz RJ, Silva E, and e Silva MR. Effects of volume resuscitation on splanchnic perfusion in canine model of severe sepsis induced by live Escherichia coli infusion. *Crit Care* 8: R221, 2004.

16. Langenberg C, Wan L, Egi M, May C, and Bellomo R. Renal blood flow in experimental septic acute renal failure. *Kidney int*69: 1996-2002, 2006.

17. Losser M-R, Forget A-P, and Payen D. Nitric oxide involvement in the hemodynamic response to fluid resuscitation in endotoxic shock in rats. *Crit care med*34: 2426-2431, 2006.

18. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, Akech SO, Nyeko R, Mtove G, Reyburn H, and Lang T. Mortality after fluid bolus in African children with severe infection. *N Engl J Med*364: 2483-2495, 2011.

19. Martin C, Boisson C, Haccoun M, Thomachot L, and Mege J-L. Patterns of cytokine evolution (tumor necrosis factor-alpha and interleukin-6) after septic shock, hemorrhagic shock, and severe trauma. *Crit care med*25: 1813-1819, 1997.

20. Mulder MF, van Lambalgen AA, and van den Bos GC. The fall of cardiac output in endotoxemic rats cannot explain all changes in organ blood flow: a comparison between endotoxin and low venous return shock. *Shock* 5: 135-140, 1996.

21. Natanson C, Danner RL, Reilly JM, Doerfler ML, Hoffman WD, Akin GL, Hosseini JM, Banks SM, Elin RJ, and MacVittie T. Antibiotics versus cardiovascular support in a canine model of human septic shock. *Am J Physiol Heart Circ Physiol*259: H1440-H1447, 1990.

22. Oi Y, Åneman A, Svensson M, Ewert S, Dahlqvist M, and Haljamäe H. Hypertonic saline-dextran improves intestinal perfusion and survival in porcine endotoxin shock. *Crit care med*28: 2843-2850, 2000.

23. Ottosson J, Dawidson I, Brandberg A, Idvall J, and Sandor Z. Cardiac output and organ blood flow in experimental septic shock: effect of treatment with antibiotics, corticosteroids, and fluid infusion. *Circ shock* 35: 14-24, 1991.

24. Passmore MR, Fung YL, Simonova G, Foley SR, Dunster KR, Diab SD, Tung J-P, Minchinton RM, McDonald CI, and Anstey CM. Inflammation and lung injury in an ovine model of extracorporeal membrane oxygenation support. *Am J Physiol Lung Cell Mol Physiol*311: L1202-L1212, 2016.

25. Rahal L, Garrido AG, Cruz Jr RJ, Silva E, and Poli-de-Figueiredo LF. Fluid replacement with hypertonic or isotonic solutions guided by mixed venous oxygen saturation in experimental hypodynamic sepsis. *J Trauma Acute Care Surg*67: 1205-1212, 2009.

26. Rivers EP, Coba V, Visbal A, Whitmill M, and Amponsah D. Management of sepsis: early resuscitation. *Clin chest med*29: 689-704, 2008.

27. Sadaka F, Juarez M, Naydenov S, and O'Brien J. Fluid Resuscitation in Septic Shock The Effect of Increasing Fluid Balance on Mortality. *J intensive care med*0885066613478899, 2013.

28. Schmidhammer R, Wassermann E, Germann P, Redl H, and Ullrich R. Infusion of increasing doses of endotoxin induces progressive acute lung injury but prevents early pulmonary hypertension in pigs. *Shock* 25: 389-394, 2006.

29. Smith III E, Slivjak M, Egan J, Gagnon R, Arleth A, and Esser K. Fluid resuscitation improves survival of endotoxemic or septicemic rats: Possible contribution of tumor necrosis factor. *Pharmacology* 46: 254-267, 1993.

30. Somell A, Sollevi A, Suneson A, Riddez L, and Hjelmqvist H. Beneficial effects of hypertonic saline/dextran on early survival in porcine endotoxin shock. *Acta anaesthesiol scand*49: 1124-1134, 2005.

31. Suffredini AF, Fromm RE, Parker MM, Brenner M, Kovacs JA, Wesley RA, and Parrillo JE. The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med*321: 280-287, 1989.

32. Ungerstedt U, and Rostami E. Microdialysis in neurointensive care. *Curr pharm des*10: 2145-2152, 2004.

33. Vallet B, Lund N, Curtis SE, Kelly D, and Cain SM. Gut and muscle tissue PO2 in endotoxemic dogs during shock and resuscitation. *J App Physiol*76: 793-800, 1994.

34. Van Lambalgen A, Bronsveld W, Van Den Bos G, and Thijs L. Distribution of cardiac output, oxygen consumption and lactate production in canine endotoxin shock. *Cardiovasc res*18: 195-205, 1984.

35. Vincent J, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall J, and Payen D. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med*34: 344, 2006.

Figure Legends:

Figure 1: Schematic representation of experimental protocol. Endotoxemia was induced with a four hour infusion of LPS, followed by 12 hours of monitoring. Haemodynamic support with vasopressors was initiated at the beginning of the last hour of the endotoxin infusion.

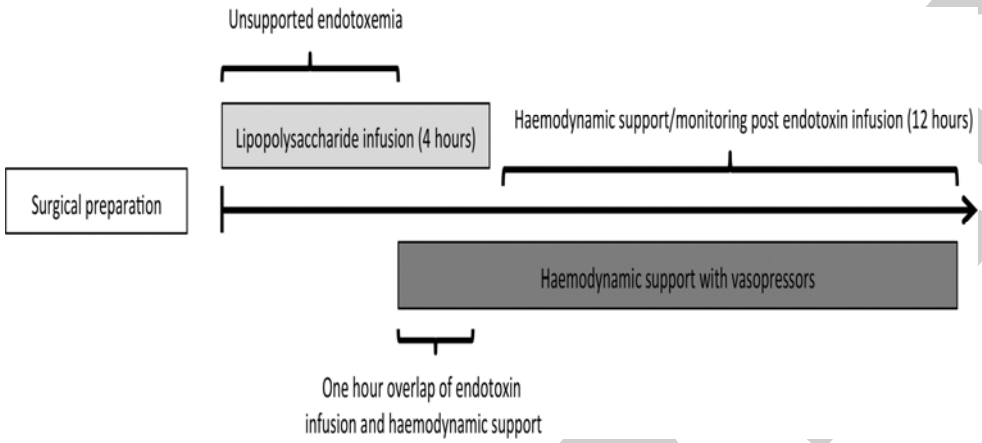


Figure 2: Cardiac index (A), mean arterial pressure (B), systemic vascular resistance index (C) and mean pulmonary artery pressure (D) during the first three hours of endotoxin infusion prior to commencement of haemodynamic support. Data are reported as means ± SEM. Both mean arterial pressure and systemic vascular resistance decreased during endotoxemia ($p<0.05$). Cardiac index was not significantly changed ($p=0.0845$). Mean pulmonary artery pressure increased during endotoxemia ($p=0.01$).

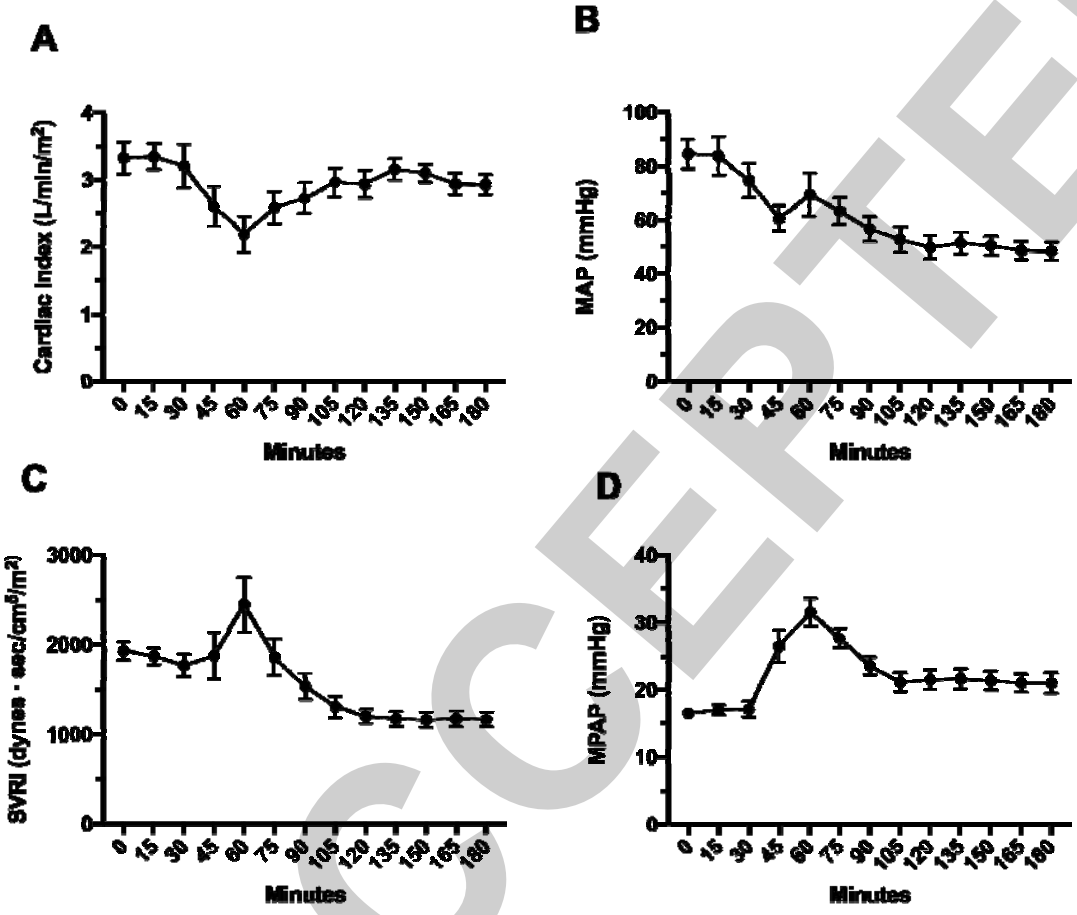


Figure 3: Mean noradrenaline (A, mcg/min) and vasopressin (B, units/hr) infusion rates after initiation of haemodynamic support (titrated to maintain MAP 60-65mmHg). Data reported as mean ± SEM.

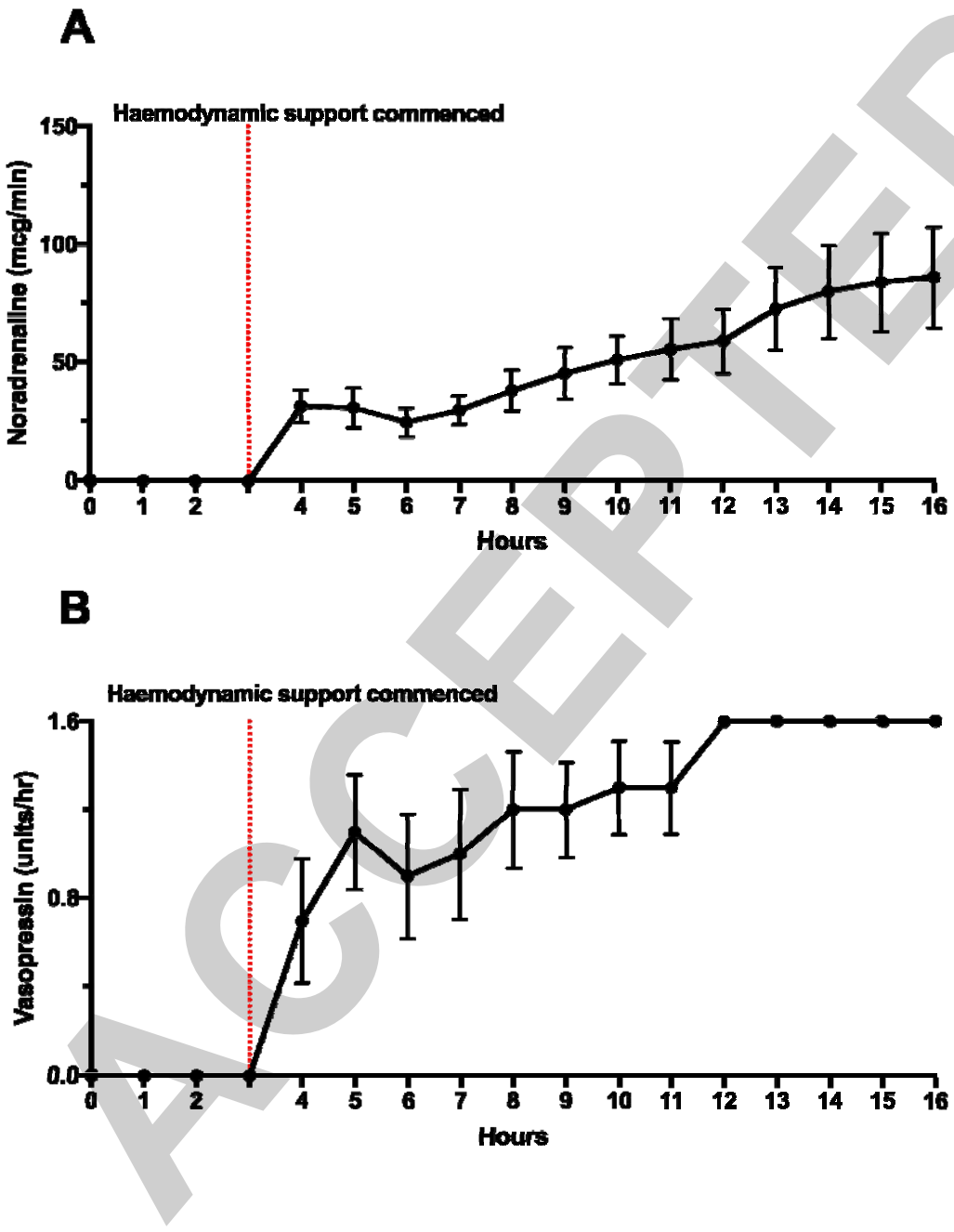


Figure 4: A) Hb, B) WBC and C) creatinine over the course of the study. Timepoints are baseline (insertion of central line), start of endotoxin infusion (SE), Start of haemodynamic support, end of endotoxin infusion and beginning of monitoring period (0), 1 hour post endotoxin infusion, 6 hours post endotoxin infusion and 12 hours post endotoxin infusion. Data is represented as mean with $\pm$ SEM. * indicates timepoint with $p<0.05$ compared to timepoint SE.

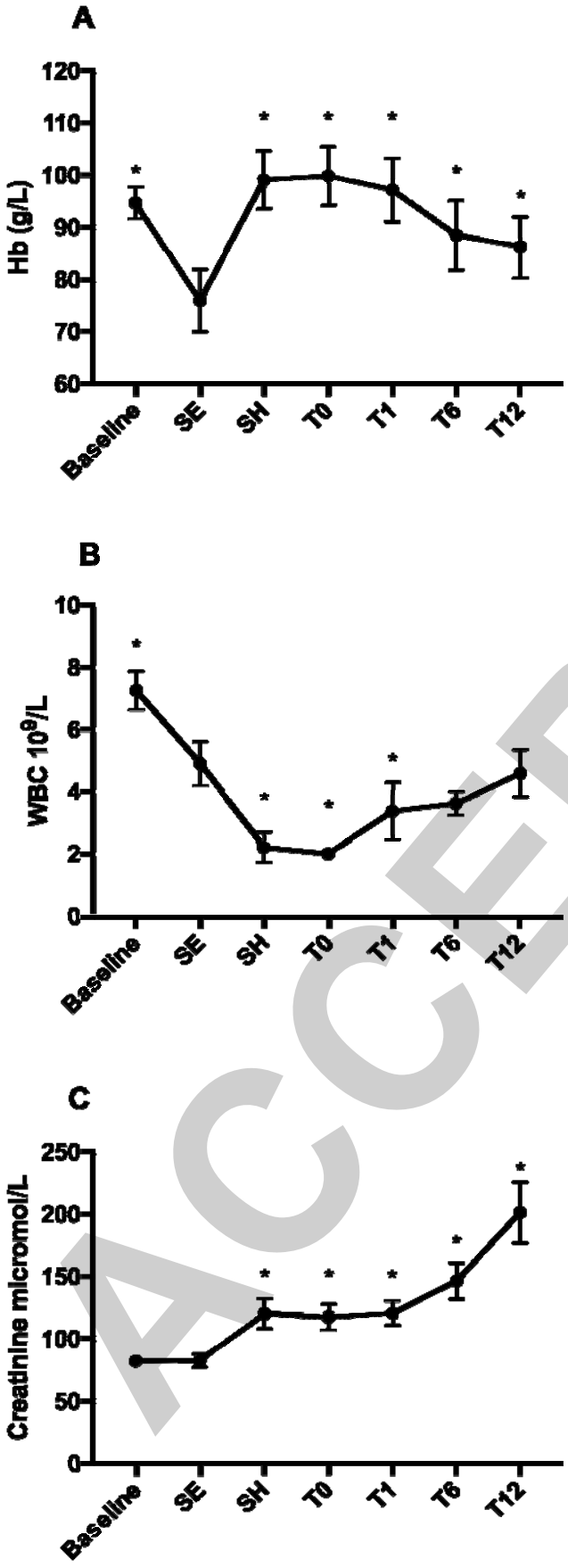


Figure 5: Serum pro-inflammatory cytokines. Timepoints are baseline (insertion of central line), start of endotoxin infusion (SE), Start of haemodynamic support, end of endotoxin infusion and beginning of monitoring period (0), 1 hour post endotoxin infusion, 6 hours post endotoxin infusion and 12 hours post endotoxin infusion. Data is represented as mean with $\pm$ SEM. * indicates timepoint with $p<0.05$ compared to timepoint SE.

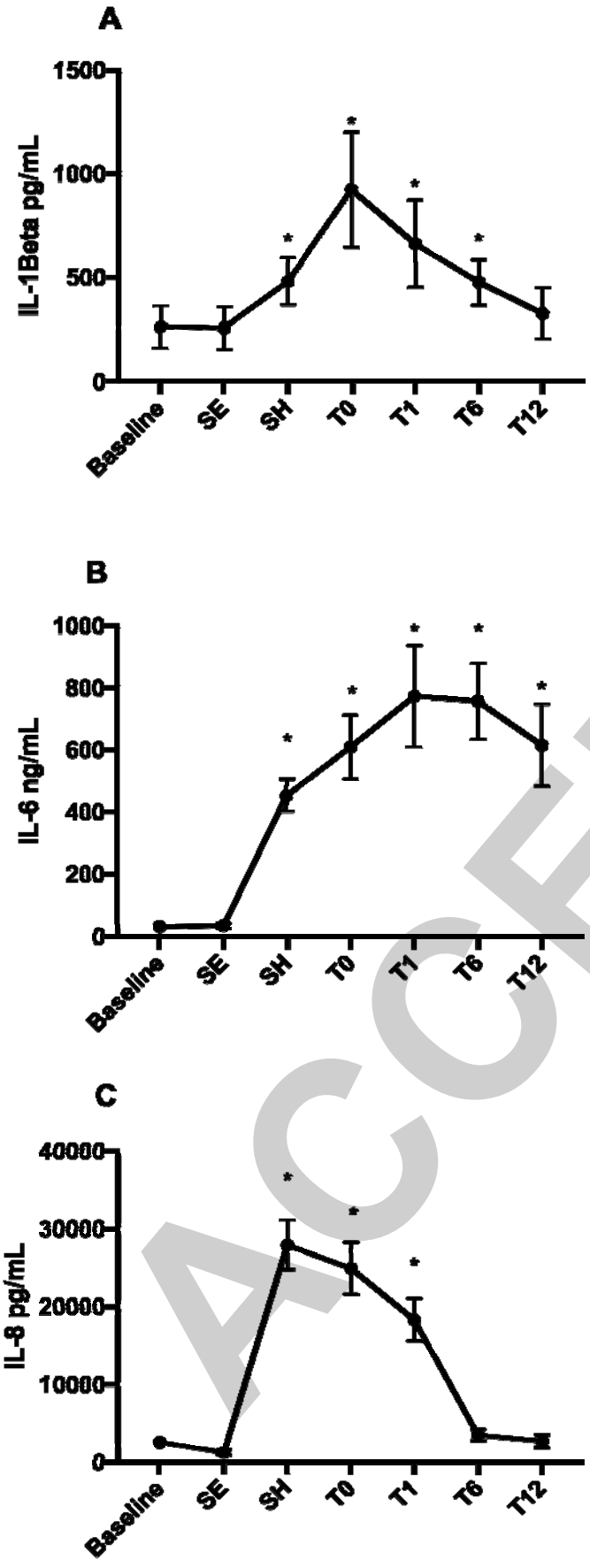
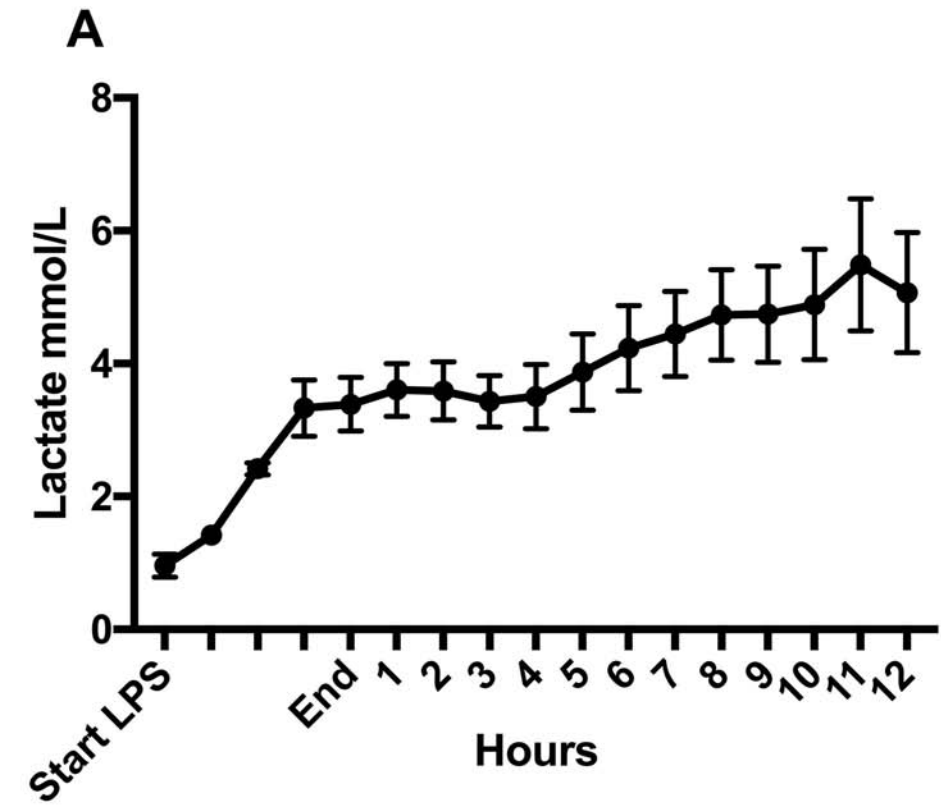

A
IL-1Beta pg/mL
1500
1000
500
0
Baseline
SE
SH
T0
T1
T6
T12
B
IL-6 ng/mL
1000
800
600
400
200
0
C
IL-8 pg/mL
40000
30000
20000
10000
0

Figure 6: A) Serum lactate and B) base excess over the study. Data is represented as means ± SEM.



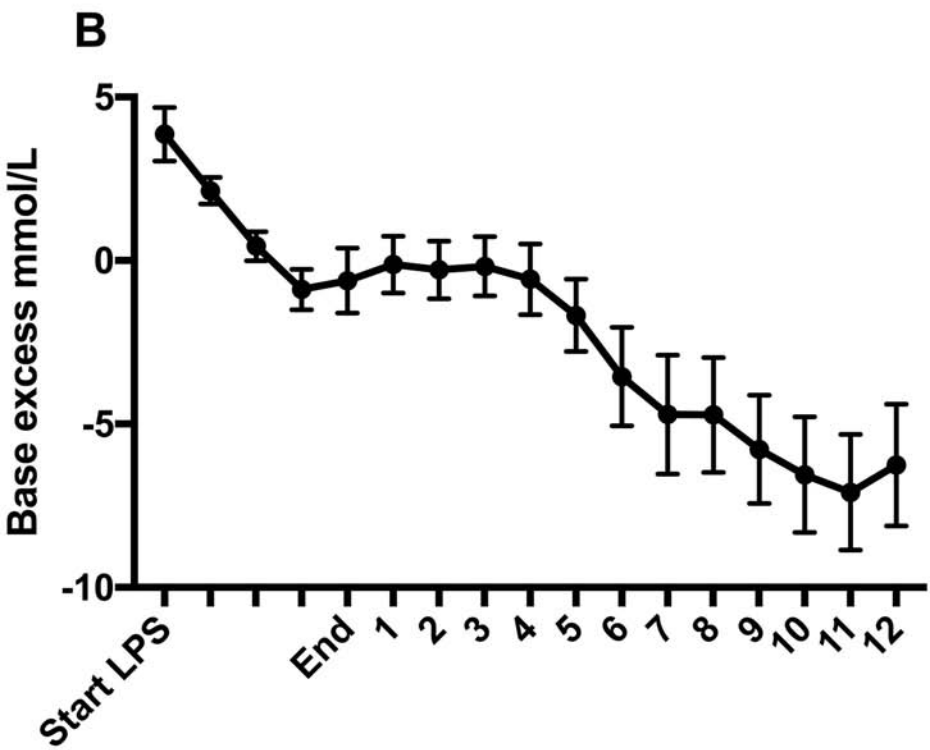


Figure 7: Mean lactate/pyruvate ratios in the brain (A), heart (B), kidney (C) and liver (D). Data reported as mean ± SEM. Dotted line at 25 indicating the upper limit of normal for the lactate/pyruvate ratio. Only the heart and liver had a significant trend in the logistic regression analysis with the heart ratio increasing heart and the liver decreasing over the monitoring period.

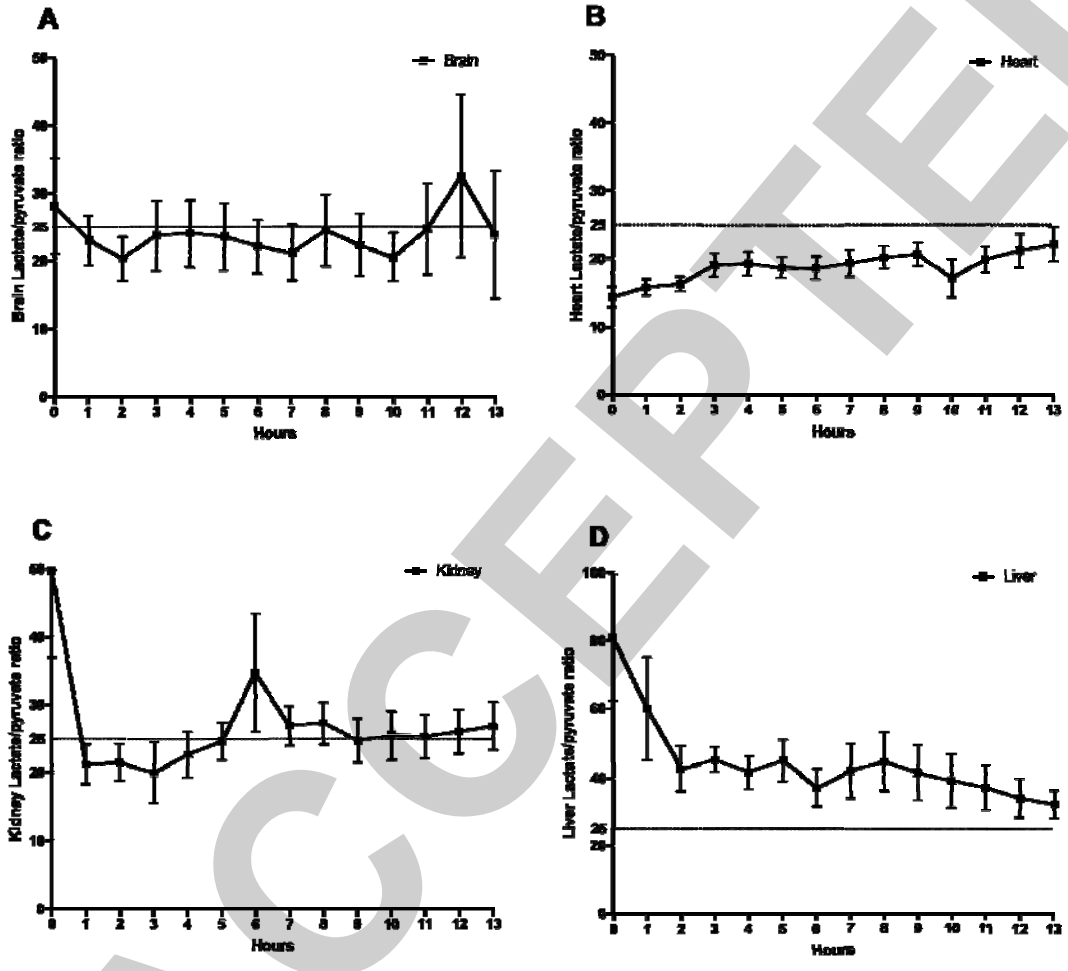


Table 1: Measured physiological parameters represented as means and 95% CI.

	(Baseline) Start of endotoxin infusion	Prior to initiation of haemodynamic support	End of monitoring period
Temperature (Degrees C^o)	38.21 ± 0.95	38.7 ± 0.96	40.08 ± 0.57
Cardiac Index ($L/min/m^2$)	3.33 ± 0.56	2.89 ± 0.36	5.0 ± 2.39
MAP (mmHg)	84.5 ± 12.8	49 ± 8.03	60.88 ± 2.3
SvO2 (SaO2)	66.88 ± 12.26	68.63 ± 13.85	76.13 ± 12.1
CVP (mmHg)	5 ± 3.21	5.63 ± 2.39	7.25 ± 2.76
MPAP (mmHg)	16.5 ± 2.1	21 ± 4.3	22.88 ± 2.8
SVRI (dyne $s/cm^5/m^{2)}$	1931 ± 241	1198 ± 176	979.5 ± 339

Table 2: Mean values for Hb, WBC and creatinine. Start of endotoxin infusion was taken as the reference value for comparison. * indicates Wald p value <0.05 compared to values at the beginning of the endotoxin infusion.

	Baseline	Start of endotoxin infusion (ref)	End of endotoxin infusion	End of monitoring period
Hb(g/L)	95 ± 6*	76 ± 14.2	100 ± 8*	86 ± 10*
WBC (10^9/L)	7.3 ± 1.3*	4.9 ± 1.7	4.4 ± 1.3*	4.6 ± 1.6
Creatinine(micromol/L)	81.9 ± 10.2	82.4 ± 12.7	117.4 ± 13.5*	201 ± 37.5*

Table 3: Serum pro-inflammatory cytokines. Data represented as means and 95% confidence intervals. Start of endotoxin infusion was taken as the reference value for comparison. * indicates Wald p value <0.05 compared to values at the beginning of the endotoxin infusion.

	Baseline	Start of endotoxin infusion (ref)	Start haemodynamics support	End of endotoxin infusion	1 hour	6 hours	12 hours
IL-1Beta pg/ml	261.9 ± 247.2	255.3 ±247.8	481.8 ±271.7*	922.5 ± 658.2*	662.4 ±496.9*	475.3 ± 258.8*	326.5 ± 290
IL-6 ng/ml	32 ±16.8	34.8±17.8	454.6 ± 120*	611.1 ± 244*	774.9 ±385.7*	759.1 ±288.4*	617.5 ± 313.3*
IL-8 pg/ml	2595 ± 1150	1196.5 ± 849.5	27981.5 ± 7646*	24977.9 ± 7942*	18290.4 ± 6569*	3425.8 ± 1786	2658.1 ± 1914

Table 4: Mean log of lactate/pyruvate ratios of each organ while animals were haemodynamically supported with mean log values exported to actual mean values.

Site	Mean log L/P ratio (95% CI)	Mean L/P ratio (95% CI)
Arterial	3.08 (3.02-3.15)	21.8 (20.4-23.3)
Brain	3.00 (2.89-3.12)	20.1 (18.0-22.6)
Heart	2.94 (2.87-3.01)	18.9 (17.7-20.2)
kidney	3.12 (3.02-3.21)	22.5 (20.4-24.9)
liver	3.63 (3.54-3.72)	37.9 (34.6-41.5)