Vital Organ Blood Flow During Hyperdynamic Sepsis*

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Objectives: To develop a nonlethal model of hyperdynamic sepsis, and to measure vital organ blood flows in this setting.

Design: Randomized crossover animal study.

Setting: Animal laboratory of university-affiliated physiology institute.

Subjects: Seven Merino cross sheep.

Interventions: Surgical implantation of transit-time flow probes around sagittal sinus and circumflex coronary, superior mesenteric, and left renal arteries, and of an electromagnetic flow probe around the ascending aorta. After recovery, randomization to either 6 h of observation under normal conditions (control) or 6 h of observation after the induction of hyperdynamic nonlethal sepsis (sepsis), with each animal crossing over to the other treatment after a 2-week interval.

Measurements and main results: Injection of *Escherichia coli* induced nonlethal hyperdynamic sepsis within 5 to 6 h with hypotension (mean arterial pressure [± SD], 85 ± 7 mm Hg vs 69 ± 8 mm Hg), increased cardiac output (4.0 ± 0.9 L/min vs 7.2 ± 1.2 L/min), tachycardia (60 ± 10 beats/min vs 160 ± 15 beats/min), fever, oliguria, and tachypnea. Compared to control animals, hyperdynamic sepsis increased renal (330 ± 101 mL/min vs 214 ± 75 mL/min), mesenteric (773 ± 370 mL/min vs 516 ± 221 mL/min), and coronary (54 ± 24 mL/min vs 23 ± 10 mL/min) blood flow (p < 0.05). There was no significant change in sagittal sinus flow. Despite increased coronary flow, myocardial contractility decreased (800 ± 150 L/min/s vs 990 ± 150 L/min/s). Despite increased mesenteric and renal blood flow, there was hyperlactatemia (0.5 ± 0.1 mmol/L vs 1.9 ± 0.3 mmol/L); despite increased renal blood flow, all experimental animals acquired oliguria (160 ± 75.3 mL/2 h vs 50.2 ± 13.1 mL/2 h) and increased serum creatinine levels (0.07 ± 0.02 mmol/L vs 0.11 ± 0.02 mmol/L).

Conclusions: Injection of *E coli* induced hyperdynamic nonlethal sepsis. During such hyperdynamic sepsis, blood flow to heart, gut, and kidney was markedly increased; however, organ dysfunction developed. We speculate that global ischemia may not be the principal mechanism of vital organ dysfunction in hyperdynamic sepsis.

Key words: blood flow; brain; cerebral circulation; coronary circulation; gut; heart; kidney; sepsis

Abbreviations: dF/dt = myocardial contractility index; MAP = mean arterial pressure

Septic shock is a major cause of morbidity and mortality in intensive care. The majority of deaths are late and due to multiple organ dysfunction. The etiology of organ dysfunction is complex and poorly understood, but the adequacy of global organ blood flow is thought to be central to the development of organ failure in sepsis.
Evidence regarding global organ flow in sepsis is contradictory, with some studies showing decreased and others increased or unchanged flows. The nature of the septic model is probably the most important reason for these conflicting results. Many studies use a bolus of endotoxin to induce sepsis; however, this often results in a hypodynamic state with reduced cardiac output and BP. This hypodynamic state is in contrast with what is measured clinically in human sepsis, where marked systemic vasodilation and increased cardiac output are typical. Therefore, although many animal studies show a reduction in regional blood flows, their relevance to human sepsis is questionable.

Hyperdynamic models that simulate human sepsis are more difficult to develop; therefore, there is a paucity of data on organ blood flow during hyperdynamic mammalian sepsis. Accordingly, we have developed a model of sepsis that results in a reproducible, nonlethal hyperdynamic state similar to human sepsis. Using this model, we have investigated the effects of hyperdynamic sepsis on global vital organ blood flow and now report our findings.

**Materials and Methods**

**Animal Preparation**

The institutional Animal Ethics Committee approved this study. Seven Merino cross ewes weighing between 35 kg and 45 kg were procured for chronic instrumentation. The animals underwent four separate operative procedures. For all procedures, anesthesia was induced with sodium thiopentone (15 mg/kg) for endotracheal tube placement (cuffed size 10). Maintenance anesthesia was by means of oxygen/air/isoflurane (1 to 2%). Fractional inspired oxygen was altered to maintain Pao2 at approximately 100 mm Hg, and ventilation was controlled to maintain Paco2 at approximately 40 mm Hg.

The first procedure was oophorectomy and carotid loop creation. After 2 to 3 weeks, a sagittal sinus flow probe was placed using a stereotactic device (Howard Florey Institute; Melbourne, Australia). A longitudinal incision was made over the vertex to expose the bregma. A craniotomy was then performed slightly anterior to the lambdoid suture to expose the dura mater and superior sagittal sinus. Two longitudinal incisions were made either side of the sinus for placement of the flow probe (4 mm; Transonic Systems, Ithaca, NY). The flow probe was covered with silicone film and secured in place with dental acrylic. The overlying skin was closed. This technique has been previously described and validated as an accurate index of cerebral blood flow in sheep.

One week later, a left-sided thoracotomy was performed. The pericardium was opened, and a transit-time flow probe (3 mm; Transonic Systems) was placed around the circumflex artery and an electromagnetic flow probe (In Vivo Metrics; Healdsburg, CA) around the ascending aorta.

After 2 weeks, a left-sided flank incision was made and retroperitoneal dissection was performed to expose the superior mesenteric and left renal arteries. Transit-time flow probes (6 mm and 4 mm, respectively; Transonic Systems) were placed around these arteries. The animals were allowed to recover for approximately 3 weeks. The use of chronically implanted transit-time flow probes has been previously validated.

The transit-time flow probes were connected to a Transonic T201CD flow meter via a four-channel sequential scanner (TM04; Transonic Systems). The electromagnetic flow probes were activated by a Biotronex flowmeter (Biotronex; Kensington, MD). The output voltage of the electromagnetic flowmeter was reset to zero using an auto zero circuit during a portion of diastole when blood flow in the ascending aorta is assumed to be zero. A separate circuit measured the first differential of the upstroke of systole (myocardial contractility index [dF/dt]) at each beat. Approximately 1 month after implantation, the electromagnetic flow probes were calibrated in vitro against thermodilution over a range of cardiac output values. Dobutamine was used to increase cardiac output from approximately 4 to 9 L/min.

The day before each experiment, a carotid loop arterial Tygon catheter (Estec; Melbourne, Australia) [inner diameter, 1.0 mm; outer diameter, 1.7 mm] and internal jugular venous polythene catheter (inner diameter, 1.2 mm; outer diameter, 1.7 mm) were placed to measure arterial and central venous pressures. The cannulae were connected to pressure transducers (TDXIII; Cobe; Lakewood, CO) tied to the wool on the back of the sheep. A correction factor was added in the data collection program to compensate for the height of the transducers above the heart. A urinary catheter was inserted for urine flow measurements and sample collection.

Analog signals (mean arterial pressure [MAP], central venous pressure, cardiac output, dF/dt, regional flows) were collected using a PC486 data acquisition system using custom software written at the Howard Florey Institute. Data were collected at 100 Hz for 10 s at 10-min intervals throughout the experimental protocol.

**Protocol and Measurements**

The sheep were held and studied in metabolic cages, with free access to food and water. The day after catheter insertion, after a 2-h observation period, sheep were randomized to either the "control" or "sepsis" arm of the study. Sepsis was induced by an IV bolus injection of 3 × 10^8 cfu of *Escherichia coli* (isolated from blood cultures of a patient who recovered from septic shock). The bacteria were grown from stock kept at 70°C and incubated overnight in broth. The culture medium was then adjusted by turbidometry to give the correct concentration of bacteria, and this was confirmed subsequently with colony counts.

In the control group, IV normal saline solution (approximately 1 mL/kg/h) was administered by infusion as fluid maintenance, and measurements began immediately after the end of the 2-h prerandomization period. In the sepsis group, IV normal saline solution (2 to 3 mL/kg/h) was administered as fluid maintenance, and measurements began with the onset of the hyperdynamic sepsis. The onset of hyperdynamic sepsis, for the purpose of this study, was prospectively defined by the simultaneous presence of the following criteria:

1. 50% increase in heart rate, 
2. > 50% increase in cardiac output, and 
3. > 10% decrease in MAP.

The onset of hyperdynamic sepsis typically occurred within 6 h of *E coli* injection. MAP, cardiac output, heart rate, sagittal sinus flow, coronary flow, mesenteric flow, and renal flow were measured continuously. Urinary flow was measured and sampled every 2 h for analysis (Model 3CI Osmometer; Advanced Instruments; Needham Heights, MA). Arterial blood samples for analysis of arterial blood gases, serum urea, creatinine, and electrolytes (Beckman; Brea, CA) were obtained at 0 min, 30 min, 60 min, 180 min, and 360 min during the observation period.

No fluid boluses, isotropic support, mechanical ventilation, or antibiotics were administered; however, all sheep made a full recovery.
recovery within 48 h. The animals were conscious and not sedated for the duration of the experiment. After approximately 2 weeks, the sheep were crossed over to the other arm of the study.

Statistical Analysis

Data are presented as mean ± SD. Comparison of central hemodynamics between the pre-E coli state and the septic state were performed comparing mean values for each period and using the Wilcoxon signed-rank test. Comparisons of hemodynamics, biochemistry, and regional blood flows between the control period and the septic period were performed by comparing the area under the curves as described by Matthews et al16 and the Wilcoxon signed-rank test; p < 0.05 was considered statistically significant.

RESULTS

Induction of Hyperdynamic Sepsis

The administration of E coli induced hyperdynamic sepsis with delayed onset at 5 to 6 h after injection: tachycardia (60 ± 10 beats/min vs 160 ± 15 beats/min, p < 0.05), increased cardiac output (4.0 ± 0.9 L/min vs 7.2 ± 1.2 L/min, p < 0.05), and hypotension (85 ± 7 mm Hg vs 69 ± 8 mm Hg, p < 0.05) [Fig 1, 2]. During such hyperdynamic state, the right atrial pressure was maintained between 2 mm Hg and 5 mm Hg as IV fluids were administered according to protocol.

Comparison of Regional Flow (Sepsis vs Control)

During the 6 h of observation in the above-mentioned septic state, animals had increased coronary (54 ± 24 mL/min vs 23 ± 10 mL/min, p < 0.05), mesenteric (773 ± 370 mL/min vs 516 ± 221 mL/min, p < 0.05), and renal (330 ± 101 mL/min vs 214 ± 75 mL/min, p < 0.05) flows when compared to the 6 h of the control period for the same animals. These changes were mostly dependent on regional vasodilatation (increased conductance). There were no changes in sagittal sinus flow (Fig 3).

This hyperdynamic circulatory state and the observed increase in organ blood flow were associated with the onset of impaired myocardial contractility (800 ± 150 L/min/s vs 990 ± 150 L/min/s), hyperlactatemia (1.9 ± 0.5 vs 0.5 ± 0.1, p < 0.05), oliguria (160 ± 75 mL/2 h vs 50 ± 13 mL/2 h, p < 0.05), and increased serum creatinine (0.07 ± 0.01 mmol/L vs 0.11 ± 0.03 mmol/L, p < 0.05), when compared to the 6-h control period, thus simulating some of the typical biochemical and functional markers of organ dysfunction seen in human sepsis (Fig 4).

DISCUSSION

Multiple organ dysfunction is a major and often lethal complication of septic shock.1 The pathogenesis of this dysfunction is unclear. Inadequate global blood flow to vital organs is considered pivotal in its development.2 Such belief stems from the observation that in several experimental studies of septic shock, global organ blood flow is decreased.3,5

Figure 1. Graph showing the effect of a single IV bolus of E coli on systemic hemodynamics. Approximately 6 h following the bolus, there was a decrease in MAP and an increase in heart rate and cardiac output. The line demonstrates the time of E coli bolus administration, while the enclosed rectangle demonstrates the period of time during which the regional circulations were measured.
Unfortunately, these studies typically used a bolus of endotoxin to induce septic shock leading to a hypodynamic circulation (reduced cardiac output and BP).\(^8,9\) In contrast, it has been repeatedly demonstrated that human septic shock is characterized by a normal or elevated cardiac output.\(^10–13\) Therefore, it is questionable whether the changes in global organ blood flow observed during experimental hypodynamic sepsis bear any relationship to human sepsis. In response to this important and yet unresolved issue, we have developed a hyperdynamic model of sepsis and have used it to measure global vital organ flow under conditions that reproduce many of the features of human sepsis.

Several important observations emerged from our study. First, coronary blood flow and coronary conductance were increased indicating vasodilatation of the coronary circulation. Despite increased coronary blood flow, there was a decrease in myocardial performance, as demonstrated by the reduction in dF/dt, an index of ventricular contractility. Our observations confirm that sepsis can induce myocardial dysfunction.\(^17\) Despite such dysfunction, cardiac output is increased, indicating that the clinical significance of such myocardial impairment is probably mild. Our findings also suggest that such dysfunction is unlikely to be due to global ischemia. In fact, the coronary circulation has been investigated in both hypodynamic and hyperdynamic septic states with conflicting results. In studies using a hypodynamic model, coronary blood flow was reduced\(^5\); however, in hyperdynamic models, coronary blood flow was increased.\(^17–20\) Small cohort studies\(^21,22\) in humans confirm that global myocardial ischemia is unlikely in human sepsis.

The second major finding was that global mesenteric blood flow was also increased secondary to marked vasodilatation (increased mesenteric conductance). The splanchnic circulation in sepsis has been extensively investigated; however, most experimental studies\(^4,7,23–26\) employed a hypodynamic model and showed marked splanchnic vasoconstriction. In hyperdynamic sepsis, however, blood flow to the mesenteric bed might be maintained or increased.\(^27\) The direct measurement of mesenteric blood flow is not possible in human beings. The splanchnic and renal circulations are the major source of lactate metabolism.\(^28\) Our animals acquired

![Figure 2. Comparison of systemic hemodynamics and dF/dt during the 6-h observation period. BP, cardiac output, total peripheral conductance, heart rate, and contractility were all significantly different (p < 0.05). All data are presented as mean ± SE. ■ septic, △ control.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21998/)

hyperlactatemia, while global mesenteric and renal blood flows were markedly increased. These findings do not support the view that the hyperlactatemia of hyperdynamic sepsis is secondary to hypoperfusion of lactate-metabolizing organs.

The third major finding of our study was that global renal blood and renal artery conductance increased, indicating regional vasodilatation. The response of the renal circulation to sepsis has been extensively investigated with contradictory results. In hypodynamic models, there was a reduction in renal blood flow in line with the reduction in cardiac output.3,7 In studies27,29,30 using hyperdynamic models of sepsis, the findings were variable. Despite a 50% increase in renal blood flow, there was an increase in serum creatinine and oliguria, suggesting a decrease in glomerular filtration rate. The rapid onset of such dysfunction, in the face of increased blood flow, suggests that global ischemia may not be responsible for the early phases of the development of septic acute renal failure. Indeed our findings are consistent with the intrarenal hemodynamic effects of efferent arteriolar vasodilatation.

The fourth major finding of our study was that cerebral blood flow remained unchanged during the hyperdynamic phase of sepsis; however, the cerebral blood flow to cardiac output ratio fell markedly, indicating that the increased cardiac output was essentially distributed to other organs. The cerebral circulation has been poorly investigated during ex-
Experimental hyperdynamic sepsis. The complex vascular anatomy of the brain makes it difficult to isolate vessels that do not also contribute to extracranial blood flow. In sheep, in particular, the rete mirabile can direct up to 59% of carotid blood flow to extracranial structures31; hence, simply measuring carotid blood flow is inaccurate. In animal sepsis and in septic humans, the cerebral circulation has been investigated using microspheres, transcranial and carotid artery Doppler ultrasonography or the $^{133}$Xe washout method, and the results have been highly variable.32–35

**Limitations**

Our model does not completely reproduce severe human sepsis, as none of the experimental animals died; however, in this model, all three major criteria for a hyperdynamic circulation were present, and there was no confounding effect of immediately preceding surgical intervention or sedation. Due to the complexity of the preparation (8 weeks of staged surgery), the loss of animals would have been logistically untenable and ethically questionable. We also administered IV fluids, which might have assisted in ensuring the hyperdynamic response. Our model is unique in that it is the first to have all the following features: simultaneous and continuous measurement of four vital circulations and systemic hemodynamics, use of a large mammal and bacteremia (instead of endotoxemia), induction of the systemic inflammatory response syndrome, hypotension and increased cardiac output, and avoidance of the confounding effects of sedation and recent surgery.

Our assessment of organ function was limited. However, this is the first model of severe sepsis that combines a hyperdynamic circulation, the continuous measurement of blood flow to four major vital organs, and the measurement of some clinical markers of organ function.

Sampling of venous effluent from vital organs was not performed, thus leaving some uncertainty about whether the increased blood flow was associated with an increase in oxygen extraction. However, the increased level of invasiveness associated with such cannulation is associated with thrombosis of the venous system and would have required periodic sacrifice of experimental animals, which was logistically untenable.

In conclusion, this study describes a model of hyperdynamic sepsis that mimics human sepsis. During such hyperdynamic sepsis, blood flow to heart, gut, and kidney increased; however, signs of organ dysfunction developed despite more-than-adequate organ blood flow. We speculate that global ischemia may not be the principal mechanism of vital organ dysfunction in mammalian hyperdynamic sepsis.

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