Tissue oxygen saturation is positively correlated with oxygen delivery and cardiac output in a canine hemorrhagic shock and resuscitation model

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OBJECTIVE
To determine if tissue oxygen saturation (StO₂) correlates with oxygen delivery (DO₂) and/or cardiac output (CO) in a canine hemorrhagic shock model.

ANIMALS
8 healthy purpose-bred dogs.

METHODS
Dogs were anesthetized, and hemorrhagic shock was induced by withdrawing up to 60% of total blood volume, targeting a mean arterial pressure (MAP) of 40 mm Hg. The withdrawn blood was returned to the patient in 2 equal aliquots. Data was collected at 4 time points: 10 minutes after MAP was stabilized under anesthesia (time point (TP)-1), 10 minutes after up to 60% of blood volume was removed to target a MAP of 40 mm Hg (TP2), 10 minutes after the return of 50% of shed blood (TP3), and 10 minutes after the return of the remaining 50% of shed blood (TP4). Total blood volume withdrawn, StO₂, CO, heart rate, and MAP were recorded, and DO₂ was calculated at each TP.

RESULTS
Mean StO₂ significantly decreased between TP1 (77.8% [± 9.54]) and TP2 (44.8% [± 19.5]; P < .001 vs TP1). Mean StO₂ increased to 63.1% (± 9.85) at TP3, but remained significantly lower compared to TP1 (P = .002). There was no difference between mean StO₂ at TP4 (82.5% [± 12.6]) versus TP1 (P = .466). StO₂ has a strong, positive correlation to both CO (r = 0.80; P < .001) and DO₂ (r = 0.75; P < .001).

CLINICAL RELEVANCE
A decrease in StO₂ may be used in conjunction with physical examination findings and diagnostic parameters to support a diagnosis of shock. The return of shed blood was correlated with increases in StO₂, DO₂, and CO, suggesting that StO₂ may be used as a marker of adequate resuscitation.

Keywords: tissue perfusion, shock, heart rate, blood pressure, cardiac output

Shock is commonly diagnosed in both human and veterinary emergency rooms and is defined as inadequate cellular energy production due to impaired tissue perfusion, which results in insufficient oxygen delivery (DO₂) to meet oxygen demand.¹⁻³ There are many classifications of shock; however, hypovolemic shock due to hemorrhage is the most commonly diagnosed in human emergency rooms.³

Prompt recognition of hemorrhagic shock is imperative to guide therapy and prevent mortality in the emergency setting. By simplifying the definition of shock to a state where DO₂ does not meet tissue oxygen demand, direct measurement of cardiac output (CO) with a pulmonary arterial catheter would be ideal to diagnose hemorrhagic shock. However, this is not accessible in most facilities nor clinically feasible in critically ill patients given its invasive nature. Without this direct measurement, clinicians rely on a combination of history and evaluation of perfusion parameters, including mentation, heart rate, pulse quality, mucous membrane color, capillary refill time, and core-toe-web temperature gradient, to diagnose shock. Low blood pressure is also commonly used to support a diagnosis of shock. However, perfusion parameters and blood pressure
may be minimally affected in hypovolemic shock due to neurohormonal compensatory processes that can result in increased CO, heart rate, and systemic vascular resistance. Shock index, the ratio of heart rate to systolic blood pressure, has also been used as a marker of shock in human and veterinary patients, with recent studies\(^4\)–\(^10\) citing a shock index of > 1 as an accurate marker of shock when compared to controls. In addition to physical examination, diagnostic tests including lactate and base excess, may be used to support a diagnosis of shock. Recently, studies\(^1\)\(^1\)\(^1\) have shown that the caudal vena cava collapsibility measured using point-of-care ultrasound can be used clinically to evaluate volume status and resuscitation in shock.

Near-infrared spectroscopy (NIRS) measures oxygenated and deoxygenated hemoglobin in tissues, reflecting the balance of tissue DO\(_2\) and consumption.\(^1\) In humans, this tool has been shown to predict the need for blood product administration in trauma victims that otherwise appear cardiovascularly stable.\(^1\)\(^2\) Multiple veterinary studies have published data evaluating the use of this technology in dogs, establishing reference intervals in healthy dogs.\(^3\) Comparing tissue oxygen saturation (StO\(_2\)) in dogs on room air versus nasal oxygen supplementation,\(^4\)\(^4\) and documenting the positive correlation between StO\(_2\) and DO\(_2\) in hemorrhagic shock models and naturally occurring shock patients.\(^1\)\(^5\)–\(^1\)\(^7\) To date, no studies have been performed evaluating the correlation of StO\(_2\), DO\(_2\), and CO.

The purpose of this study was to determine if StO\(_2\) was correlated with DO\(_2\) and CO and could therefore be used as a marker of shock in dogs. The second purpose was to determine if DO\(_2\) could be used as a marker of resuscitation. We hypothesized that StO\(_2\) would be positively correlated with DO\(_2\) and CO and could therefore be used as a marker of shock and resuscitation.

**Methods**

**Animals**

Eight purpose-bred spayed female Beagles were used in this study. Each dog was determined to be healthy based on physical examination, complete blood count, and biochemistry panel. All procedures were approved by the IACUC at Colorado State University. This study was performed from September to December 2021.

**Anesthesia and instrumentation**

Dogs were premedicated with hydromorphone (0.1 mg/kg, IM), and an IV catheter was placed in a cephalic vein. Following preoxygenation, general anesthesia was induced with propofol (5 to 10 mg/kg, IV) to effect. A cuffed endotracheal tube was placed and connected to an anesthetic circle system, and general anesthesia was maintained with isoflurane vaporized in 100% fraction of inspired oxygen. Dogs were spontaneously breathing throughout the study.

Dogs were instrumented with ECG, pulse oximetry, invasive blood pressure monitoring, esophageal temperature probe, and end-tidal CO\(_2\) via sidestream sampling for the duration of general anesthesia. Normothermia was maintained using a forced-air warming device and heated water blankets.

After induction and placement of anesthetic monitoring, the StO\(_2\) probe was placed in a clipped 5 X 5 cm region over the sartorius muscle on the upward-facing leg. A 20-gauge over-the-needle catheter was placed in a dorsal metatarsal artery, and a 16-gauge over-the-needle catheter was placed in a jugular vein. A 7.5F Swan-Ganz catheter (Biosensors International Inc, Japan, Tokyo) was placed using a flow-directed technique via the jugular vein, and the location in the pulmonary artery was confirmed via pressure waveform analysis. All catheters were placed using aseptic technique.

**StO\(_2\), CO, and DO\(_2\) measurements**

Measurement of StO\(_2\) was performed using a noninvasive portable near-infrared spectroscopy (ODISsey Tissue Oximeter, ViOptix Inc), which has been validated in animal models.\(^1\)\(^8\),\(^1\)\(^9\) Prior to data collection in each dog, the machine was calibrated per manufacturer instructions. Per manufacturer recommendations, any StO\(_2\) measurement with a signal quality < 80% was not reported.

Measurement of CO via thermodilution was performed per manufacturer guidelines. A temperature probe was placed in an ice bath of syringes. A 5-mL bolus of 0.9% NaCl was injected into the proximal port of the Swan-Ganz catheter, and CO was calculated by system software (Hemosphere Advanced Monitoring Platform, Edwards LifeSciences). All CO measurements were obtained in duplicate with 3 to 5 minutes between measurements, and the average of measurements was used for statistical analysis.

After completion of the study, DO\(_2\) was calculated using the following equation: DO\(_2\) = CO \(\times\) \{(1.34 X Hb X SpO\(_2\)) + (0.003 X PaO\(_2\))\}, where SpO\(_2\) is peripheral oxygen saturation of hemoglobin as measured by pulse oximetry and PaO\(_2\) is alveolar partial pressure of oxygen.

**Experimental design**

Once each dog was anesthetized and instrumented, the mean arterial pressure (MAP) was stabilized at 70 to 80 mm Hg for 10 minutes before time point (TP)-1 measurements were obtained. If necessary, dobutamine (0.5 to 3 μg/kg/min, IV), phenylephrine (0.5 to 2 μg/kg/min, IV), and/or an IV bolus of crystalloids (3 to 20 mL/kg) were used to stabilize blood pressure within the desired range. Two patients received an intervention (dobutamine, dobutamine) to achieve target blood pressure for TP1. These were discontinued prior to blood removal.

A low CO state was induced by removing the dog’s blood from the jugular catheter over 20 minutes, targeting a MAP of 40 mm Hg or until 60% of the dog’s total blood volume (blood volume, 90 mL/kg) was removed, whichever occurred first. Once a MAP of 40 mm Hg was reached, blood withdrawal was stopped. Blood was stored in blood collection bags containing citrate phosphate dextrose adenine. After 10 minutes, TP2 measurements were
collected. If MAP had increased from 40 mm Hg during the 10-minute wait time, no further blood was withdrawn. Fifty percent of the withdrawn blood was returned back to the dog over 15 minutes, and after 10 minutes, the final TP4 measurements were obtained. After completion of the study, dogs were deinstrumented and allowed to recover from general anesthesia. All dogs survived without complication and participated in further research for a limited time prior to being adopted.

CO, arterial blood pressure, heart rate, SpO₂, body temperature, StO₂, and end-tidal CO₂ were recorded at all TPs. An arterial blood gas was performed at all TPs. Hemoglobin and PaO₂ from the arterial blood gases were used to calculate DO₂.

Statistical methods
Descriptive statistics were compiled for each variable and timepoint. In order to investigate changes over time, a mixed model was fit separately for each response variable. Specifically, TP1–4 were included as a fixed effect. Dogs were included as a random effect to account for the repeated measures design. The Dunnett method was used to compare downstream TPs to baseline (TP1). Residual diagnostic plots were used to evaluate model assumptions of normality and equal variance. For CO and DO₂, log transformation was used to satisfy model assumptions. Normally distributed variables are reported as mean (± SD), and non-normally distributed variables are reported as median (range). Correlations between variables were calculated accounting for repeated measures on subjects. 20 Statistical analyses were performed by commercial software (SAS Institute Inc). A value of \( P < .05 \) was considered statistical evidence of a difference.

Results
Eight healthy female spayed purpose-bred Beagles were enrolled in this study. The mean age was 4.8 years (± 1.0). The mean body weight was 8.8 kg (± 1.56).

The mean blood volume withdrawn was 36.3 mL/kg (± 11.6). The MAP at TP1, TP2, TP3, and TP4 was 74.1 mm Hg (± 7.4), 49.5 mm Hg (± 13.7), 63.5 mm Hg (± 13.3), and 71.4 mm Hg (± 8.88), respectively.

The median CO at TP1 was 2.7 L/min (range, 1.2 to 3.8). This decreased significantly at TP2 (0.6 L/min; range, 0.5 to 1.5; \( P < .001 \) vs TP1; Tables 1 and 2).

The median CO was 2.0 L/min at TP3 (range, 1 to 2.6;

<table>
<thead>
<tr>
<th>Variable</th>
<th>TP1</th>
<th>TP2</th>
<th>TP3</th>
<th>TP4</th>
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<tbody>
<tr>
<td>StO₂ (%)</td>
<td>77 (± 9.54)</td>
<td>44.8 (± 19.5)</td>
<td>63.1 (± 9.85)</td>
<td>82.5 (± 12.6)</td>
</tr>
<tr>
<td>DO₂ (mL/min/m²)</td>
<td>460.7 [227.7–686.9]</td>
<td>121.6 [82.1–246.6]</td>
<td>311.1 [194.7–415.0]</td>
<td>373.7 [266.1–908.0]</td>
</tr>
<tr>
<td>CO (L/min)</td>
<td>2.7 [1.2–3.8]</td>
<td>0.6 [0.5–1.5]</td>
<td>2.0 [1.0–2.6]</td>
<td>2.5 [1.8–5.3]</td>
</tr>
<tr>
<td>Blood removed (mL/kg)</td>
<td>36.3 (± 11.6)</td>
<td>18.4 (± 5.98)</td>
<td>18.4 (± 5.98)</td>
<td>18.4 (± 5.98)</td>
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<tr>
<td>SpO₂</td>
<td>96.0 (± 2.62)</td>
<td>95.9 (± 2.53)</td>
<td>96.9 (± 1.69)</td>
<td>96.5 (± 1.66)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>74.1 (± 7.4)</td>
<td>49.5 (± 13.7)</td>
<td>63.5 (± 13.3)</td>
<td>71.4 (± 8.88)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.5 (± 2.03)</td>
<td>13.6 (± 2.18)</td>
<td>11.9 (± 1.75)</td>
<td>11.1 (± 1.63)</td>
</tr>
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Table 1—Median [range] or mean (SD) values for cardiovascular variables of tissue oxygen saturation (StO₂), global oxygen delivery (DO₂), cardiac output (CO), blood removed, peripheral oxygen saturation of hemoglobin as measured by pulse oximetry (SpO₂), mean arterial pressure (MAP), and hemoglobin concentrations (Hb) at time points (TPs).

Figure 1—Median and range oxygen delivery (DO₂) values at time point (TP)-1 (baseline), TP2 (maximum blood removed), TP3 (50% shed blood returned), and TP4 (100% shed blood returned). \( P \) values comparing TP1 versus TP2, TP3, and TP4 are < 0.001, 0.047, and 0.98, respectively (Table 1). Asterisks represent statistically significant differences between TPs.

Figure 2—Median and range tissue oxygen saturation (StO₂) values at TP1 (baseline), TP2 (maximum blood removed), TP3 (50% shed blood returned), and TP4 (100% shed blood returned). \( P \) values comparing TP1 versus TP2, TP3, and TP4 are < 0.001, 0.002, and 0.47, respectively (Table 1). Asterisks represent statistically significant differences between timepoints.
The median DO₂ at TP1 was 460.7 mL/min/m² (range, 227.7 to 686.9). This decreased significantly at TP2 to 121.7 mL/min/m² (range, 82.1 to 246.6) when compared to TP1 (P < .001; Tables 1 and 2; Figure 1). Median DO₂ was significantly different at TP3 at 311.1 mL/min/m² (range, 194.8 to 415) when compared to TP1 (P = .047; Figure 1). Median DO₂ was not significantly different at TP4 (373.7 mL/min/m² [range, 266.1 to 908]) compared to TP1 (P = .98; Figure 1). Due to machine error in reading hemoglobin, 1 dog did not have a calculable DO₂ at TP3 and was therefore left out of statistical analysis regarding DO₂.

The mean StO₂ at TP1 was 77.8% (± 9.54). This decreased significantly at TP2 to 44.8% (± 19.5) when compared to TP1 (P < .001; Tables 1 and 2; Figure 2). Mean StO₂ increased to 63.1% (± 9.85) at TP3 but remained significantly lower compared to TP1 (P = .002). There was no difference between the mean StO₂ at TP1 and TP4 (82.5% ± 12.6; P = .466).

Figure 3—Repeated measures correlation curves between tissue oxygen saturation (StO₂) and cardiac output. Each line style and symbol pair indicate the results for 1 dog. The overall correlation is r = 0.80; P < .001.

Figure 4—Repeated measures correlation curves between tissue oxygen saturation (StO₂) and global oxygen delivery (DO₂). Each line style and symbol pair indicate the results for 1 dog. The overall correlation is r = 0.75; P < .001.
There was a strong positive correlation between \( \text{StO}_2 \) and \( \text{CO} \) \((r = 0.80; \ P < .001; \text{Figure 3})\) and a strong positive correlation between \( \text{StO}_2 \) and \( \text{DO}_2 \) \((r = 0.75; \ P < .001; \text{Figure 4})\).

**Discussion**

This study’s hemorrhagic shock and resuscitation model in healthy dogs found strong positive correlations between \( \text{StO}_2 \) and \( \text{DO}_2 \) and \( \text{StO}_2 \) and \( \text{CO} \), supporting our hypothesis. \( \text{StO}_2 \), as measured by a NIRS device, offers an objective, noninvasive, portable, easy-to-use method to assess patients at presentation and during resuscitation.\(^{13,15}\) The median \( \text{StO}_2 \) decreased significantly after removal of the maximal amount of blood in this study (TP2), suggesting that \( \text{StO}_2 \) can be used in conjunction with physical examination and diagnostic parameters as a noninvasive tool to support a diagnosis of shock. The \( \text{StO}_2 \) increased after 50% of the shed blood was returned to the patient and increased further when 100% of the shed blood was returned, suggesting that \( \text{StO}_2 \) can be used as a marker of resuscitation in hemorrhagic shock. The baseline (TP1) \( \text{StO}_2 \) was comparable to prior studies in dogs with and without supplemental oxygen.\(^{13,14}\)

While not a focus of this study, \( \text{CO} \) at all TPs was reflective of acute blood loss and replenishment back to baseline after resuscitation. Baseline \( \text{CO} \) values were within normal reference ranges reported in a veterinary study examining CO in a hemorrhagic shock model.\(^{21}\) \( \text{CO} \), as measured by thermodilution, was used as the gold standard in this study for determining shock and resuscitation. However, it requires placement of a pulmonary arterial catheter, which has been associated with increased mortality or morbidity in human studies, raising concern for its safety and efficacy in critically ill patients.\(^{22}\)

The positive correlation between \( \text{StO}_2 \) and \( \text{DO}_2 \) in this study is consistent with a prior study\(^{17}\) with a similar design but using a different NIRS machine. The prior study\(^{17}\) documented a stronger correlation between \( \text{StO}_2 \) and \( \text{DO}_2 \) \((r = 0.97; \ P = .005)\), which could be due to a larger sample size of 14 Beagles compared to 8 in the present study or due to the use of a different NIRS machine. The present study included TP3 after 50% of shed blood was returned, which allowed for documentation of trends in \( \text{CO}, \text{StO}_2, \) and \( \text{DO}_2 \) at multiple levels of hypovolemia, which was not included in the previous study.

Despite withdrawing blood until the target \( \text{MAP} \) of 40 mm Hg was reached, the mean \( \text{MAP} \) at TP2 was 49.5 mm Hg. The discrepancy between the target \( \text{MAP} \) and TP2 \( \text{MAP} \) is likely due to activation of endogenous compensatory mechanisms, such as activation of the renin-angiotensin-aldosterone system, fluid shifts, baroreceptor activation, and peripheral vasoconstriction during the 10-minute wait time between cessation of blood withdraw and TP2 measurements.

The limitations of this study include the small sample size of 8 dogs. Additionally, only female dogs were used in this study. The deleterious effects of extrapolating data from one sex to another has been highlighted in human literature, citing sex as a biologic variable.\(^{25}\) A recent study\(^{24}\) documented significant differences in stroke volume index and \( \text{CO} \) between healthy males and females at rest using different measures of echocardiography. There are currently no studies comparing \( \text{CO} \) between male and female dogs. These were all clinically normal dogs prior to undergoing general anesthesia. Blood loss in this model was performed under controlled conditions while receiving 100% inspired fraction of inspired oxygen, which is not representative of the clinical presentation of hemorrhagic shock. Additionally, this model did not induce trauma or tissue damage, which is commonly associated with hemorrhagic shock. Trauma and tissue damage may induce a significant inflammatory response, which may impact hemodynamic coherence.\(^{25}\) Inhaled anesthetics will contribute to some level of cardiovascular disruption. In addition, the absence of conscious fear, pain, and/or anxiety is eliminated under general anesthesia, which could impact heart rate, \( \text{CO} \), and subsequently \( \text{DO}_2 \) by nature of the equation. Individual variance in pigmentation and body condition score may contribute to the wide range of \( \text{StO}_2 \) readings.\(^{13}\) In our study, the NIRS probe was placed on an area of white skin in all dogs.

In conclusion, in this canine fixed-pressure hemorrhagic shock model, there was a strong positive correlation between \( \text{StO}_2 \) and \( \text{DO}_2 \) and between \( \text{StO}_2 \) and \( \text{CO} \). There was a significant decrease in \( \text{StO}_2 \) after the withdrawal of blood, suggesting that it may be used in conjunction with physical examination and diagnostic parameters to support a diagnosis of shock. In addition, \( \text{StO}_2 \) returned to baseline values after the return of all shed blood, suggesting that it may be used as a marker of resuscitation in hemorrhagic shock. Further studies in larger groups and clinical patients are warranted.

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**Disclosures**

The authors have nothing to disclose. No AI-assisted technologies were used in the generation of this manuscript.

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**References**