Microcirculatory Effects of Hyperviscous Hemoglobin-based Fluid Resuscitation in a Canine Model of Hemorrhagic Shock

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By:
Ann Peruski B.S. D.V.M
Graduate Program in Veterinary Clinical Sciences

The Ohio State University
2010

Thesis Committee:
Edward S. Cooper, Advisor
Amy L. Butler
Robert L. Hamlin
Abstract

Hemorrhagic shock is a common and life-threatening complication of severe trauma in humans and dogs alike. Massive blood loss leads to inadequate oxygen delivery to tissues secondary to tissue hypoperfusion and decreased oxygen carrying capacity. Physiologic compensation for hemorrhagic shock includes increases in heart rate, cardiac output, and global vasoconstriction, however, these mechanisms can be overwhelmed if shock goes untreated, resulting in circulatory collapse and patient death.

Until recently, determining the severity of hemorrhagic shock as well as the response to treatment could only be performed by evaluating macrovascular variables (such as heart rate and arterial blood pressure), venous blood gas analysis (such as lactate and base excess) or invasive tissue monitoring (such as tissue pO₂ and tissue pCO₂). While these parameters are very helpful in gauging overall response to shock resuscitation, they do not necessarily reflect perfusion of the microcirculation. The microcirculation is controlled by both systemic and local factors. Ongoing microvascular compromise can persist despite normalization of systemic parameters with resuscitative efforts. As such, direct assessment of the microcirculation might serve to better gauge the severity of hemorrhagic shock and help guide resuscitation. This has been made possible with sidestream dark field microscopy (SDF) which allows for direct imaging
and real-time assessment of the microcirculation. This device is a non-invasive, handheld instrument that directly images and records videos of the microcirculation, allowing off-line analysis of several microvascular flow parameters.

Although numerous studies have been performed in search of the ideal resuscitation fluid for hemorrhagic shock (crystalloid, colloids, artificial oxygen carriers, blood components), no clear benefit of one fluid over another has been identified. In addition, administration of these fluids following hemorrhagic shock results in a decrease in whole blood and plasma viscosity, which may contribute to ongoing microvascular vasoconstriction secondary to decreased nitric oxide production. Under normal conditions, production of nitric oxide, a potent vasodilator, is dependent on activation of the constitutive nitric oxide synthase. This enzyme is induced by shear stress on the endothelium- a process highly dependent on plasma viscosity. Recent evidence suggests that hyperviscous fluids may provide an advantage over traditional shock therapy, by restoring blood viscosity and endothelial shear stress; thereby preserving functional capillary density and improving microvascular perfusion.

Hemoglobin-based oxygen carrying (HBOC) fluids have been developed to promote oxygen delivery to the tissues while avoiding the potential complications of blood component transfusions. Despite their potential benefits, HBOCs have been associated with a number of adverse effects in humans, owing primarily to their ability to
scavenge nitric oxide and promote vasoconstriction. These side effects can be manifested in humans as an increased risk of myocardial infarction or hypertension following administration of an HBOC. In this experiment, we hypothesized that resuscitation from hemorrhagic shock with a viscosity-enhanced HBOC solution would restore all macrovascular parameters to baseline, as well as preserve microvascular flow (as seen with SDF) by limiting vasoconstriction as compared to a standard HBOC solution.

The test fluid (hyperHBOC) was made by addition of 0.3% alginate, an inert seaweed polymer, to a standard HBOC solution with a resultant average viscosity of 4.93 cP. The standard HBOC solution (sHBOC) served as the control fluid and had an average viscosity of 1.37 cP. Twelve dogs (male conditioned foxhounds) were randomly assigned to either the hyperHBOC group (n=6) or the sHBOC group (n=6). The dogs were placed under general anesthesia and instrumented with central venous, cephalic, mesenteric venous, carotid and dorsal pedal arterial catheters for blood sampling, induction of hemorrhagic shock, and cardiovascular monitoring. They were splenectomized to prevent the hemodynamic effects of splenic contraction and allowed a 30 minute equilibration period prior to induction of hemorrhage. The dogs were then bled via the carotid artery catheter until a mean arterial pressure of 35-40 mmHg was achieved, and then maintained in this shock state for a period of 60 minutes. At the end
of this period each dog received a 30 ml/kg bolus of either hyperHBOC or sHBOC over 20 minutes for resuscitation.

Data were collected at baseline, after the end of the shock period and 30, 60, 120, and 180 minutes post-resuscitation. At each time point, macrovascular parameters (vital signs, arterial, central and mesenteric venous blood gases, lithium dilution cardiac output) as well as videos of the microcirculation of the buccal mucosa and jejunal serosa were collected. The videos were archived for offline analysis at a later date. A 2-way repeated measures ANOVA was used to assess differences between time points and treatment groups. Single comparisons were performed for selected variables with a Student’s t-test. Intraobserver variability was calculated for analysis of the microvascular videos.

The results showed no significant differences between groups at baseline and after the shock period, suggesting that the two groups achieved an equivalent degree of shock severity. Following resuscitation, both groups showed return to baseline for macrovascular variables. There were no significant differences seen between the groups with regard to microvascular parameters for both buccal mucosa and jejunal serosa, and following resuscitation, the values were not significantly different from baseline. Dogs receiving hyperHBOC had higher oxygen extraction ratios and decreased central venous saturation compared to those receiving sHBOC at several time points. The hyperHBOC
group also had higher systemic vascular resistance indices than the control dogs after resuscitation; however, the values were not significantly different from baseline.

Based on these results, there is no apparent benefit to administration of hyperHBOC over a sHBOC solution for the resuscitation of hemorrhagic shock in dogs. However, these findings should be interpreted in the context of the potential limitations of the study, including the effects of anesthesia and small sample size, among others. Use of SDF microvascular imaging provided insight into the microcirculatory effects of hemorrhagic shock and resuscitation, and may prove to be a useful tool in the clinical setting.
Dedication

Thank you to Dr. Edward Cooper, my program mentor and inspiration for this project. Without his encouragement and dedication, this paper would not have been possible. Also thank you to Dr. Amy Butler for her continued support for this project and assistance with manuscript preparation, and Dr. Robert Hamlin, who served on my thesis committee. Finally, thank you to Dr. Shane Bateman, who was instrumental in my decision to pursue a career in critical care medicine.

A big thank you to my family and friends as well, for their love, support, and guidance through the past five years, I could not have done it without them.
Acknowledgements

Special thanks to Yukie Ueyama for her technical assistance during this project, and to Laboratory 0137 for use of their equipment.

Funding for this study provided through the Canine Research Fund, an institutional grant provided by the College of Veterinary Medicine, The Ohio State University, Columbus, OH

The Oxyglobin® used in this experiment was donated by BioPure, Cambridge, MA

The central venous catheters used in this experiment were donated by Mila International, Erlanger, KY
VITA

2005………………..B.S. Veterinary Medicine, Michigan State University
2005………………..Doctor of Veterinary Medicine, Michigan State University
2006………………..Internship in Small Animal Medicine and Surgery,
                      The Ohio State University
2007………………..Clinical Instructor in Small Animal Critical Care and
                      Shelter Medicine, The Ohio State University
2010………………..Residency in Small Animal Emergency and Critical Care
                      Medicine, The Ohio State University

FIELD OF STUDY

Major Field: Veterinary Clinical Sciences
Table of Contents

Page

Abstract..................................................................................................................ii
Dedication...............................................................................................................vi
Acknowledgements................................................................................................viii
Vita...........................................................................................................................ix
List of Figures.........................................................................................................xii
List of Tables..........................................................................................................xiv

Chapter 1: Literature Review

1.1 Pathophysiology of hemorrhagic shock.......................................................1
1.2 The microcirculation.......................................................................................6
1.3 Hemodynamic monitoring.............................................................................10
1.4 Treatment of hemorrhagic shock.................................................................16
1.5 Hyperviscous resuscitation..........................................................................22

Chapter 2: The Experiment

2.1 Introduction..................................................................................................32
2.2 Materials and methods.................................................................35
2.3 Results.........................................................................................41
2.4 Discussion and conclusion..........................................................43
Bibliography.......................................................................................60
Appendix A: List of proprietary materials...........................................69
Appendix B: List of cardiovascular equations.......................................71
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>25</td>
</tr>
<tr>
<td>1.2</td>
<td>25</td>
</tr>
<tr>
<td>1.3</td>
<td>26</td>
</tr>
<tr>
<td>1.4</td>
<td>26</td>
</tr>
<tr>
<td>1.5</td>
<td>27</td>
</tr>
<tr>
<td>1.6</td>
<td>27</td>
</tr>
<tr>
<td>1.7</td>
<td>28</td>
</tr>
<tr>
<td>1.8</td>
<td>28</td>
</tr>
<tr>
<td>1.9</td>
<td>29</td>
</tr>
<tr>
<td>1.10</td>
<td>30</td>
</tr>
<tr>
<td>1.11</td>
<td>30</td>
</tr>
<tr>
<td>2.1</td>
<td>50</td>
</tr>
<tr>
<td>2.2</td>
<td>50</td>
</tr>
<tr>
<td>2.3</td>
<td>51</td>
</tr>
<tr>
<td>2.4</td>
<td>51</td>
</tr>
</tbody>
</table>
2.5 Perfused vessel density- buccal mucosa.....................................................53
2.6 Perfused vessel density- jejunal serosa.....................................................53
2.7 Microvascular flow index- buccal mucosa..................................................54
2.8 Microvascular flow index- jejunal serosa ..................................................54
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1: Classification of hemorrhagic shock</td>
<td>31</td>
</tr>
<tr>
<td>2.1: Central venous blood gas data</td>
<td>54</td>
</tr>
<tr>
<td>2.2: Arterial blood gas data</td>
<td>55</td>
</tr>
<tr>
<td>2.3: Mesenteric venous blood gas data</td>
<td>56</td>
</tr>
<tr>
<td>2.4: Macrovascular variables</td>
<td>57</td>
</tr>
<tr>
<td>2.5: Microvascular variables</td>
<td>58</td>
</tr>
<tr>
<td>2.6: Single comparison variables</td>
<td>59</td>
</tr>
<tr>
<td>2.7: Intraobserver variability for video analysis</td>
<td>59</td>
</tr>
</tbody>
</table>
CHAPTER 1
LITERATURE REVIEW

1.1 Pathophysiology of hemorrhagic shock

Hemorrhage is a potentially devastating complication following severe trauma, coagulopathy, or surgical procedures. If blood loss is severe, or goes untreated, hemorrhagic shock may develop, potentially resulting in death. Even if a patient survives the initial insult, they may later develop fatal complications associated with the inflammatory response induced by the shock state.\textsuperscript{[1]} As such, prompt recognition and treatment of hemorrhage is essential to improving patient survival. Shock is a syndrome, characterized by the clinical signs of inadequate tissue perfusion, which results in tissue ischemia and leads to multiple metabolic and inflammatory consequences. During hemorrhagic shock, loss of circulating blood volume leads to tissue hypoxia, however, a significant amount of blood must be lost before clinical signs become apparent. Hemorrhage can be classified into four categories based on severity (Table 1.1): Grade I hemorrhage (<15% blood loss) will cause minimal effects in a healthy subject and may be clinically undetectable. Grade II hemorrhage (15-30% blood loss) will result in the earliest signs of shock: mild tachycardia, prolonged capillary refill time and anxiety. Grade III (30-40% blood loss) will result in tachycardia, hypotension, pale mucous membranes, oliguria, and altered mental status. Grade IV (>40-50% blood loss) hemorrhage results in severe physiological derangements, profound hypotension, and
may lead to permanent end-organ damage and death.\[1\] Decreased cardiac output and arterial pressure secondary to volume loss lead to decreased oxygen delivery (DO\(_2\)) and increased oxygen extraction ratio (O\(_2\)ER). In normal states, changes in oxygen delivery may have little impact on tissue oxygen consumption (VO\(_2\)), referred to as the delivery independent phase. However as DO\(_2\) continues to fall past the critical point, a linear decline in VO\(_2\) will happen and anaerobic metabolism may predominate (Fig. 1.1).\[2\] This is referred to as the delivery dependent phase.

The body has several physiologic mechanisms for coping with hemorrhage (Fig. 1.2). In the initial stages of acute hypovolemia, stroke volume falls from decreased preload and baroreceptors in the aortic arch sense decreased stretch. This reflex activates the sympathetic nervous system, eventually resulting in tachycardia. Increased sympathetic tone also leads to widespread arteriolar vasoconstriction through stimulation of \(\alpha_1\) adrenergic receptors, which in turn causes increased vascular resistance. In addition, venous return to the heart is augmented by decreased venous capacitance. Blood flow to select capillary beds, such as the coronary and cerebral circulations, is preserved in the compensated stages of hemorrhagic shock. In fact, coronary vasodilation occurs under the influence of input from the aortic and carotid bodies, mediated by local nitric oxide release. As hemorrhagic shock progresses, however, these autoregulatory mechanisms can be overwhelmed and ultimately mean arterial pressure falls.\[1\]

While perfusion is maintained to the heart and brain, vasoconstriction of other capillary beds occurs. The gastrointestinal tract is a major shock organ in the dog and
hemorrhagic shock can have serious consequences.\textsuperscript{[3]} Splanchnic vasoconstriction occurs in response to angiotensin II and vasopressin release.\textsuperscript{[4-5]} Large arterioles constrict, while mucosal arterioles dilate in an effort to preserve mucosal flow, a process mediated by prostaglandins.\textsuperscript{[6]} All of these alterations in intestinal blood flow can lead to increased oxygen extraction, and altered oxidative phosphorylation.\textsuperscript{[1]} Derangements in microvascular flow to the intestinal tract may also predispose to reperfusion injury and bacterial translocation across the mucosal surface following resuscitation\textsuperscript{[1]}. These complications may predispose patients to sepsis and multi-organ dysfunction.

Multiorgan injury also occurs during hemorrhagic shock. In the liver, hyperglycemia occurs early in the shock state, secondary to gluconeogenesis and glycogenolysis under the influence of cortisol, glucagon, and catecholamines.\textsuperscript{[7]} Decreased hepatic ATP content and mitochondrial dysfunction have also been described in early hemorrhagic shock.\textsuperscript{[1]} Renal failure secondary to tubular necrosis may also occur. During shock, intracellular acidosis and renal depletion of ATP may result in failure of ATP-driven ion pumps to maintain normal urine electrolyte gradients. In addition, decreased renal perfusion during shock leads to constriction of the efferent arterioles in an effort to maintain glomerular filtration rate. Superficial cortical blood flow is also diverted to preserve medullary and deep cortical flow.\textsuperscript{[1]}

At the microcirculatory level, many alterations occur during hemorrhagic shock. As described above, the initial response to hemorrhage is constriction of large arterioles; however this response is not homogenous. Some capillaries may be constantly perfused, some may be intermittently perfused, and flow may completely cease in some capillaries.
Eventually, capillary vasodilation will occur if shock goes untreated, and is one of the signs of decompensated hemorrhagic shock.\textsuperscript{[1]}

At the cellular level, the consequences of hemorrhagic shock can induce profound damage, even at sites remote to the initial injury, and may potentially lead to multiple organ dysfunction syndrome or acute respiratory distress syndrome.\textsuperscript{[8]} Mitochondria are responsible for cellular energy production, and consume the majority of oxygen delivered to the tissues.\textsuperscript{[9]} During shock states, mitochondrial dysfunction characterized by changes in membrane potentials and uncoupling of the electron transport chain leads to inadequate energy production, and this may persist following treatment and normalization of oxygen delivery.\textsuperscript{[10]} This is known as dysoxia, and may lead to further release of inflammatory mediators and enhanced apoptosis via the intrinsic (mitochondrial) pathway.\textsuperscript{[10-12]} Direct cellular injury also occurs during hemorrhagic shock. Release of cytokines promotes neutrophil chemotaxis and activation, leading to respiratory burst and oxidative injury. In addition, inflammatory pathways are activated including COX-2 and CD-14.\textsuperscript{[13]}

 Decompensated hemorrhagic shock occurs when the body’s compensatory mechanisms are overwhelmed. This stage is characterized by vasodilation that does not respond to fluid loading or vasopressors.\textsuperscript{[2]} This phenomenon appears to be regulated in part by nitric oxide production from the inducible nitric oxide synthase (iNOS).\textsuperscript{[14]} Release of other vasoactive mediators secondary to ischemia such as bradykinin, adenosine, CO\textsubscript{2}, lactate, and inflammatory mediators (such as leukotrienes and prostaglandins) also contribute to the development of heterogeneous blood flow, overriding of compensatory mechanisms, and ultimately irreversible vasodilation.\textsuperscript{[1]} Release
of pro-inflammatory cytokines such as tumor necrosis factor alpha, macrophage inflammatory protein-1 alpha, interleukin-6, interleukin-10, macrophage-derived chemokine, nuclear factor κB and granulocyte macrophage colony stimulating factor as well as complement activation all contribute to a global inflammatory response.\cite{15-18} This pro-inflammatory state leads to activation of the coagulation pathways and formation of microvascular thrombosis. Due to the complexity of feedback loops and crosstalk between inflammatory pathways, severe hemorrhage can potentially result in widespread organ failure and disseminated intravascular coagulation (Fig 1.2). This cycle of inflammation can self-perpetuate long after the clinical signs of bleeding have abated. Prompt recognition and treatment of hemorrhagic shock as well as modulation of the inflammatory response may be very important in improving outcomes, and this presents an area of constantly evolving research and innovation.
1.2 The microcirculation

The microcirculation is composed of vessels, measuring less than 100 microns in diameter, and is responsible for nutrient delivery within various tissues. Arterioles, venules and true capillaries (<10 micron diameter) make up the microcirculation, which despite its small size, contains the largest vascular surface area in the body. The microcirculation is organized into functional units based on a feeder arteriole, smaller arterioles, the capillary bed and the venules, which eventually feed into the macrovascular venous system (figs. 1.3-1.4). Physiological shunts are also normally present, which may allow arteriolar to venular flow, completely bypassing the capillary bed. Perfusion of capillary beds is tightly regulated at the level of the arteriolar precapillary sphincter. Numerous systemic and local factors control vasoconstriction or vasodilation of the precapillary sphincter. During periods of stress or shock, sympathetic activation and release of norepinephrine leads to constriction of this sphincter by activation of junctional alpha-1 receptors and decreased perfusion of the capillary bed. Vasoconstriction is also seen following activation of the renin-angiotensin aldosterone system and subsequent release of arginine vasopressin. Some mediators of inflammation and coagulation such as thrombin and transforming growth factor-beta can also lead to microvascular vasoconstriction. Dilation of the precapillary sphincter can happen under the influence of acetylcholine, increased heat, increased carbon dioxide or lactate production, or release of vasoactive mediators such as histamine, bradykinin, and prostacyclin in health or disease states.
Local control of the microcirculation can happen by several mechanisms. There is venular-arteriolar countercurrent flow, which provides feedback from the local tissues about metabolic needs, such as local increases in lactate or carbon dioxide. This system also allows for responses that begin in the capillary, tissue, or venule to be transmitted to the arteriole and precapillary sphincter. In addition, changes in the capillary partial pressure of oxygen can also regulate flow, with decreased $p_O_2$ resulting in dilation of the precapillary sphincter as well as stimulation for nitric oxide release.

Nitric oxide is an important regulator of microcirculatory flow, serving as a potent vasodilator. Normal endothelium maintains vascular tone and has inherent expression of small amounts of nitric oxide. The enzyme responsible for the formation of physiologic amounts of nitric oxide is the constitutive nitric oxide synthase (cNOS), which is located on the endothelial surface. In pathological states, nitric oxide is produced by the inducible nitric oxide synthase (iNOS) in response to activated macrophages, endothelial cells and cytokines. In contrast to the small amounts of nitric oxide produced by cNOS, activation of iNOS will lead to massive nitric oxide production and possibly systemic vasodilation.\textsuperscript{[20]}

Microcirculatory production of nitric oxide by cNOS depends on the amount of shear stress exerted on the endothelial surface, proportional to the local blood viscosity. Briefly, normal shear stress on the arterial wall (25 dynes/cm\(^2\)) activates the cNOS in a calcium dependent cascade. L-arginine, the precursor for nitric oxide, is progressively oxidized into the active nitric oxide molecule\textsuperscript{[21]}. Once formed, nitric oxide diffuses into
local vascular endothelial cells and induces arterial vasodilation through a cGMP-mediated pathway (fig. 1.5).\textsuperscript{[20]}

Blood viscosity is an important determinant of endothelial shear stress in the microcirculation. In whole blood, red blood cell mass is responsible for generating the majority of normal viscosity, with a normal value of approximately 5 cP in the dog.\textsuperscript{[22]} Blood coursing through the microcirculation has a decreased viscosity compared to blood within large vessels, sometimes approaching a viscosity of 1-2 cP, similar to plasma. This happens due to the relative decrease in hematocrit seen in the microvasculature, which results from the Fahraeus-Lindqvist effect as well as decreased deformability of the red blood cells themselves (fig. 1.6).\textsuperscript{[23]}

During hemorrhagic shock, increases in circulating catecholamines, angiotensin II and vasopressin promote vasoconstriction, as a way to divert blood flow to the preserved circulations of the brain and heart. Following fluid resuscitation, systemic parameters (especially blood pressure) can normalize in response to volume expansion and release of these mediators is decreased. However, significant alterations in microvascular blood flow can persist.\textsuperscript{[24-26]} Loss of circulating red blood cell mass associated with hemorrhage along with shifting of water from the interstitium into the intravascular space in an attempt to augment circulating volume, results in hemodilution and decreased blood viscosity. Fluid resuscitation with traditional crystalloids and colloids, which are hypoviscous to blood, further reduces viscosity. The reduction in blood viscosity promotes vasoconstriction at the level of the microcirculation through decreased shear stress and nitric oxide release from the endothelium.\textsuperscript{[25, 27]} This microvascular
vasoconstriction can be reflected by a decrease in functional capillary density (FCD); the number of capillaries that possess red blood cell transit within a given time period.\textsuperscript{[28]} Maintaining FCD during shock resuscitation has been strongly associated with increased tissue survival in a hamster model of hemorrhagic shock.\textsuperscript{[29]}
1.3 Monitoring Shock and Resuscitation

During shock, there may be derangements of blood flow to various organs, or an inability of tissues to utilize delivered oxygen.\[30] Until recently, macrovascular parameters such as heart rate, mean arterial pressure, and cardiac output were used to guide resuscitation and assess response to therapy. These measures are relatively insensitive representatives of changes in the microcirculation as they do not relay information on tissue oxygen delivery, tissue metabolic state, or local blood flow to the various organs. For example, blood pressure is regulated through baroreceptor responses, which aim to maintain a constant mean arterial pressure over a wide range of cardiac output. Blood pressure also does not necessarily correlate with blood flow and tissue oxygenation.\[30]

Several indirect measures have been developed to better characterize the state of the microcirculation. Gastric tonometry has been introduced as a technique to assess splanchnic perfusion. Using a special catheter, gastric mucosal pCO$_2$ can be measured. This value is used as a marker for tissue perfusion, with elevated values being highly associated with local hypoperfusion, and low values indicating increased perfusion or decreased cellular activity.\[31] The pCO$_2$ values should be interpreted in relation to the blood pCO$_2$; as hyper- or hypoventilation may confound the results.\[31-32] Unfortunately, this technique is cumbersome and not gained lasting popularity. In addition to potential interference due to ventilatory status, or cellular metabolism, use of this technique requires sedation and passage of a specialized catheter into the stomach.\[31]
Tissue oxygen delivery (DO$_2$) can be calculated as the product of cardiac output * arterial oxygen content (C$_a$O$_2 = (1.34*Hb*SO$_2$) + 0.003P$_a$O$_2$). As represented by the variables, this value depends not only on blood flow, but also on hematocrit and oxygen saturation of hemoglobin. Oxygen consumption (VO$_2$) can be calculated as the (C$_a$O$_2$-C$_v$O$_2$)/C$_a$O$_2$ * cardiac output. This value approximates the amount of oxygen that is removed from the blood and utilized by the tissues, as determined by the gradient between arterial and venous pO$_2$. These values are most useful in calculating the oxygen extraction ratio: VO$_2$/DO$_2$, which represents the amount of oxygen extracted by the tissue. These equations can be affected by alterations in blood flow to the tissues, the metabolic rate of the tissue, the ability of the tissue to extract oxygen as well as decreased hematocrit or abnormal oxygen saturation of hemoglobin.

Mixed venous oxygen saturation has been used as a marker of global tissue perfusion, with decreased values associated with increased oxygen extraction by tissues. Measurement of this value requires placement of a pulmonary artery catheter which is associated with a number of potential complications.[33] Alternatively, monitoring of central venous oxygen saturation (S$_{cv}$O$_2$) through a central catheter, sampling from the cranial vena cava or right atrium, has gained favor as a lower risk technique, and is used an end point for guiding fluid resuscitation.[34] Under normal conditions, ScvO2 is closely correlated to mixed venous saturation, however, as central venous blood does not contain blood from the caudal vena cava or coronary circulation, S$_{cv}$O$_2$ does not correlate directly with mixed venous saturation under certain conditions such as septic shock.[35-36] In this shock state, the mixed venous saturation is generally lower than S$_{cv}$O$_2$, although
significant variation occurs between patients.[36] In addition, other factors such as microvascular shunting or decreased cardiac output may cause falsely elevated or decreased readings. Despite these limitations, measurement of $S_{\text{cv}}O_2$ is used clinically as part of early goal-directed therapy.[34]

Sublingual capnography is a newer, non-invasive technique for assessing mucosal blood flow and carbon dioxide production. Similar to pulse oximetry, a small clip is placed on the non-keratinized sublingual region. This technique has been validated in septic humans, but no veterinary literature is available.[37] [38] In states of decreased local blood flow, a gradient between the sublingual and arterial pCO2 can be calculated. A high gradient may reflect decreased tissue perfusion, as not enough blood flow is present to remove CO2 from the tissues and transport it to the lungs for elimination. These changes correlated well with direct imaging of the microcirculation as well as gastric tonometry in a recent study.[37]

Direct assessment of tissue pO2, pCO2 and pH is also available by use of specialized fiberoptic probes. A porcine study of hemorrhagic shock and resuscitation demonstrated decreased pO2 as measured by a subcutaneous device implanted in the inguinal region.[39] At the same time, sensors were placed on the small intestine and colon to track the changes in these tissues. During shock, increased pCO2 and pH was noted in the intestine, and these values were inversely proportional to the subcutaneous pO2. Fluid and blood administration normalized all parameters.[39] While the results of this study are encouraging, this technology is not readily available and has not been validated in other animal species.
Perhaps the best method of assessing microcirculatory flow is through direct imaging. Intravital microscopy is a method of performing real-time microvascular imaging on anesthetized or conscious animals. This modality has been extensively used with the hamster cheek pouch model, a common microvascular model.\textsuperscript{[40]} While this is an effective method of evaluating the microcirculation directly, the equipment is cumbersome and expensive, making it impractical for clinical use and limiting its applicability to research only.

Orthogonal polarization spectral imaging (OPS) is a newer modality which uses polarized light and a specialized video camera to allow real-time imaging of the microcirculation. This technology is commercially available as a handheld device. A study evaluating image quality and the ability to accurately assess the microvascular showed that OPS was able to provide similar measurements of red blood cell velocity and capillary density, but with a superior image quality compared to conventional intravital microscopy.\textsuperscript{[41]} The device has been used extensively in research, including studies of the normal human dermal microvasculature, detection of mesenteric hypoperfusion, and assessing the depth of burn injuries.\textsuperscript{[42-44]} A major limitation of this device is the potential for pressure-induced vessel compression (as direct contact between the probe and the tissue is necessary to obtain the images), to artificially lower measurement of vessel density and blood flow.\textsuperscript{[45]}

The most recent development in clinical imaging of the microcirculation has been the introduction of sidestream darkfield microscopy (SDF). This device (MicroScan)\textsuperscript{A} uses LED-based technology for imaging, and delivers a higher capillary image quality.
than OPS.\textsuperscript{[46]} The Microscan is a handheld videomicroscope that works by emitting green light (530 nm) which is absorbed by the hemoglobin of erythrocytes (fig.1.7-1.8).\textsuperscript{[28]} With the 5x objective lens, these illuminated red blood cells show up as a dark density flowing through the microcirculation, resulting in a real-time video image with X 326 magnification.\textsuperscript{[28]} Based on previously established consensus criteria, these videos can then be analyzed off-line to determine several microcirculatory parameters including total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD), and microvascular flow index (MFI).\textsuperscript{[28]} Total vessel density is derived by superimposing a grid of three equidistant horizontal and three vertical lines over the image. The TVD is calculated based on the number of vessels crossing these lines divided by the length of those lines (Fig 1.9).\textsuperscript{[28]} The PPV is a value based on the assignment of a flow category to each vessel (continuous, intermittent, or no-flow). It is calculated as $100 \times (\text{total number of vessels} - \text{[no flow + intermittent flow]})/\text{total number of vessels}$. The PVD, a value reflecting microcirculatory perfusion similar to the previously described FCD, is calculated by multiplying TVD and PPV. The analysis software was also used to determine the MFI. This value is obtained by dividing the visual field into quadrants and characterizing the flow as absent (0), intermittent (1), sluggish (2), or normal (3). The value from each quadrant is then averaged to determine the overall MFI (Fig 1.10).\textsuperscript{[28]}

Sidestream darkfield microscopy has been used extensively in human medicine\textsuperscript{[45, 47-49]} and to a limited extent in veterinary medicine\textsuperscript{[50-51]} One study in human sepsis patients demonstrated that collapse of the microcirculation was associated with decreased
tissue oxygenation and organ dysfunction.\textsuperscript{[48]} Another study used SDF imaging to compare microcirculatory changes in sepsis and severe sepsis.\textsuperscript{[52]} SDF imaging has been used successfully to demonstrate renal, hepatic, intestinal and pancreatic microcirculations, as well as during cardiopulmonary resuscitation, heart failure, and monitoring wound healing.\textsuperscript{[53-57]} SDF is also being applied to assess response to fluid therapy in shock, and improved microcirculatory flow during the resuscitation phase is associated with less organ failure after hospital admission.\textsuperscript{[58]} In dogs, SDF has been used to establish normal microcirculatory parameters (TVD, PVD, MFI, PPV) for anesthetized healthy dogs, as well as monitor microcirculatory changes in experimental hemorrhagic shock.\textsuperscript{[50-51]} A major benefit of SDF is the portable nature of the device, making the MicroScan a useful tool for both research and clinical applications. Similar to OPS, however, there are limitations to SDF technology. In order to generate diagnostic quality videos for analysis, sufficient pressure has to be applied to the tissue, while avoiding excessive compression of the microvasculature. In addition, the videos must be exported and analyzed offline, a process that can be time consuming. Some particular limitations to this technology in dogs are excessive dark oral pigmentation, salivation, the need for patient sedation/anesthesia and the effects of motion on image quality.\textsuperscript{[51]} Despite these limitations, SDF imaging with the MicroScan continues to be used extensively in both laboratory and clinical applications. Direct observation and quantification of microvascular blood flow, seems to be a promising monitoring tool for shock resuscitation, and it is likely that this technology will be used extensively both in research and clinical patients.
1.4 Treatment of hemorrhagic shock

Treatment of hemorrhagic shock is based on fluid resuscitation and restoration of circulating volume. There are numerous volume replacement strategies, including crystalloids, artificial or natural colloids, hypertonic or hyperoncotic solutions, artificial oxygen carriers, or blood products. Studies have compared various crystalloids against each other, crystalloids against colloids or blood products, and specialized fluids against their standard counterparts.\(^{[59-61]}\) Despite exhaustive research, debate remains about which fluid is superior to most effectively achieve resuscitation.

Crystalloids are commonly used in resuscitation from hemorrhagic shock, and there is no consensus if any particular crystalloid is superior. Compared to whole blood, many crystalloids are isotonic and have similar electrolyte composition, but they are all hypoviscous and hypo-oncotic. This necessitates administration of large volumes of crystalloids, as only a fraction of the dose will remain within the intravascular space. The shock dose of crystalloid is based on the amount of volume needed to replace an expected 30% blood loss (the point at which overt clinical signs of hemorrhagic shock become manifest). Due to the volume of distribution of intravenous crystalloids, administration of 2-3 times the estimated blood loss is necessary for adequate volume expansion.\(^{[62]}\) As the dog has an average blood volume of 80-90 mL/Kg, this results in a “shock dose” of crystalloid equivalent to the entire blood volume.\(^{[62]}\) Translocation of crystalloid fluids due to low oncotic pressure (COP) and increased hydrostatic pressure may contribute to tissue edema, compartment syndrome and ongoing inflammation.\(^{[60]}\) Lactated Ringer’s solution, a popular crystalloid, has been associated with increased neutrophil chemotaxis
and respiratory burst\textsuperscript{[60]} as well as increasing apoptosis in the intestine, lung, and liver.\textsuperscript{[63-64]} Due to their hypoviscosity, crystalloid therapy is unlikely to restore and may cause further reduction of FCD due to decreased plasma viscosity and shear stress on the endothelial wall secondary to decreased NO release.\textsuperscript{[24-25, 65]}

Artificial colloids, such as hetastarch and dextrans, have higher COP compared to crystalloids, and remain in the vascular space for a longer period of time. Hetastarch, a colloid commonly used in clinical practice, has several properties that contribute to prolonged intravascular dwell time. The molecular weight of hetastarch ranges from 10-3400 kD, and the larger molecules have a longer half life than the smaller molecules. In addition, the larger particles may be big enough to occlude small openings in abnormally permeable capillaries, theoretically limiting the amount of osmotes and water that could diffuse through that pore into the interstitium.\textsuperscript{[62]} They can also promote the shift of water into the intravascular space, depending on their oncotic pressure (e.g. hetastarch with a COP of 29-32 mmHg) compared to plasma (COP of 20-25 mmHg).\textsuperscript{[62]} For this reason, a smaller dose of colloid can be given in shock resuscitation compared to a crystalloid (20 mL/kg vs. 90 mL/Kg in the dog), and the relatively larger molecules make the colloid less likely to leak into the interstitium under normal conditions.\textsuperscript{[62]} Similar to the crystalloids, artificial colloids are hypoviscous to blood (2-4 cP), and may only partially restore FCD.\textsuperscript{[66]}

The hydroxyethyl starches (HES) have also been associated with adverse effects such as coagulopathy. Coagulopathy occurs by multiple mechanisms, including hemodilution and inhibition of factor VIII/vWf. One study showed a decrease of 80% in
factor VIII/vWF, and another showed a similar drop in fibrinogen\cite{67-69}. In addition, HES have direct anti-platelet effects, including decreased expression of glycoprotein IIb/IIIa complex and reduced availability of the platelet surface fibrinogen receptor\cite{67}. A recent study in dogs confirmed that prolonged platelet closure times are seen following HES administration, possibly due to hemodilution as well as direct effects on the platelets\cite{70}. Renal failure has also been documented following HES administration\cite{71-73}. Several modified hetastarch solutions are available, which attempt to mitigate some of the deleterious effects of the fluid. Decreasing the molecular weight of the particles decreases intravascular dwell time and accelerates clearance of the hetastarch molecules\cite{74}. In addition, molar substitution (mol hydroxyethyl residues per mol glucose subunit) has an impact on clearance, and thereby half-life, with higher molar substitution resulting in greater duration of effect secondary to increased accumulation of hetastarch in plasma\cite{74}. Manipulation of the C2:C6 ratio also plays a role in clearance of hydroxyethyl starches, and solutions with a lower ratio are more quickly excreted. Therefore, decreasing the C2:C6 ratio may reduce the incidence of coagulopathy secondary to HES administration\cite{74}.

Hypertonic crystalloids and crystalloid/colloid combinations have been developed to maximize volume expansion while limiting the dose of fluid administered. Administration of a hypertonic fluid, such as 7\% saline, serves to shift free water from the interstitium, red blood cells and endothelium, thus expanding the vascular volume well beyond the volume administered\cite{62}. In addition to volume expansion, hypertonic saline has several other beneficial effects in hemorrhagic shock. Hypertonic saline has
been shown to inhibit intestinal apoptosis and limit neutrophil activation and chemotaxis during shock. Hypertonic saline also dampens the inflammatory response to hemorrhagic shock by decreasing circulating levels of proinflammatory cytokines such as tumor necrosis factor-alpha, increasing circulating levels of anti-inflammatory interleukin-10, and interleukin-1beta and preventing the post-hemorrhage surge in norepinephrine that is associated with increased mortality. Similar to isotonic crystalloids, however, hypertonic saline has a relatively brief duration of action, with rapid redistribution of fluid to the extravascular space. In an effort to create a more sustained effect, administration of hypertonic saline in combination with a colloid has been used as small volume resuscitation strategy in hemorrhagic shock in dogs.

Hemoglobin-based oxygen carriers (HBOC) are a novel class of intravenous fluids designed to deliver oxygen to the tissues while avoiding the risks of transfusion reactions and infectious disease transmission that accompany transfusion of natural blood products. In nature, hemoglobin exists as a 68 kD tetramer, with four protein subunits, each containing a non-protein heme molecule (fig. 1.11). The heme molecule contains an iron (Fe^{2+}) atom bound to nitrogen, serving as the oxygen binding site. The presence of hemoglobin increases the oxygen carrying capacity of the blood substantially over dissolved oxygen alone. Hemoglobin also carries nitric oxide in the globin part of the molecule in the form of S-nitrosylhemoglobin. This molecule can dissociate back into nitric oxide during periods of local hypoxia, causing local vasodilation and thus improving oxygen delivery in the periphery. Nitric oxide binds reversibly to a specific cysteine residue in hemoglobin; the binding depends on the state (R or T) of the
hemoglobin.\textsuperscript{[80]} Given the fact that its size is close to renal threshold, free hemoglobin is readily excreted by the kidney, which leads to a brief duration of action when these solutions are administered intravenously. In addition, free hemoglobin is a potent scavenger of nitric oxide and causes vasoconstriction.\textsuperscript{[81-83]} Owing to this effect, HBOC administration has been associated with systemic hypertension, myocardial infarction, and increased mortality in human trials. Nephrotoxicity may also occur secondary to nitric oxide scavenging in the renal vasculature. Currently there are no FDA approved HBOCs for use in humans in the United States.\textsuperscript{[84-86]}

In order to circumvent these problems, newer HBOC solutions are made by polymerizing or encapsulating the hemoglobin molecules in order to minimize the effects of free hemoglobin on the endothelium. The fluid evaluated in this study, hemoglobin glutamer-200 (Oxyglobin\textsuperscript{®}B, is an ultrapurified gluteraldehyde polymerized hemoglobin solution, FDA approved for use in dogs. This fluid consists of artificial hemoglobin polymers and tetramers, which may reduce the rate of oxidation reactions and nitric oxide scavenging compared to free hemoglobin.\textsuperscript{[87]} The larger molecules lead to an increased duration of action, as the particles need to be broken down prior to renal elimination. Oxyglobin\textsuperscript{®} has a p50 (partial pressure at which 50% of hemoglobin is saturated with O\textsubscript{2}) of approximately 54 mmHg, which is higher than the normal canine p50 of 28 mmHg. This may result in increased oxygen offloading in the arterioles and metarterioles, depriving the capillaries of oxygen and potentially contributing to increased arteriolar vasoconstriction based on local feedback mechanisms.\textsuperscript{[88]} A high-affinity HBOC (MP4, PEGylated human hemoglobin) has been developed which has a p50 of 5 mmHg, and should facilitate
offloading of the oxygen into O₂-deprived capillaries and decrease oxygen-induced vasoactivity at the arteriolar level. This fluid is the only HBOC that has not been associated with increased O₂ER compared to pRBC transfusion. In addition, MP4 is naturally hyperviscous and hyperoncotic to plasma, and some of this beneficial effect may be related to increased endothelial wall shear stress and nitric oxide production. Given this information, it raises the notion that enhancing the viscosity of Oxyglobin could have a similar beneficial effect.
1.5 Hyperviscous resuscitation

A relatively recent innovation in shock fluid therapy is the development of hyperviscous solutions. These solutions are created by addition of synthetic polymers (generally alginate) which increase the viscosity of the fluid without affecting osmotic pressure.\[26\] Alginate is an inert seaweed extract which is commonly used in hyperviscous fluid resuscitation. During hemorrhage, decreased blood viscosity occurs from loss of red blood cell mass as well as dilution of the remaining blood with water from transcompartmental fluid shifting and administration of standard fluid therapy. Decreased plasma viscosity leads to decreased shear stress on the endothelial wall, and subsequently decreased nitric oxide production. The end result of decreased nitric oxide production is vasoconstriction and maldistribution of blood flow.\[^{20, 90-91}\] Decreased blood viscosity secondary to hemodilution has been associated with decreased microvascular function, and the heterogeneity of microvascular blood flow in this state may contribute to poor tissue survival.\[^{92}\] In a study of extreme hemodilution in a hamster model, the transfusion threshold could be lowered by enhancing plasma viscosity.\[^{24, 27}\] This finding has been corroborated by additional studies, both in extreme hemodilution and hemorrhagic shock. In fact, a study using a hamster model of hemorrhagic shock found that increasing plasma viscosity resulted in better microvascular flow as assessed by intravital microscopy, as well as improved arterial base excess and O$_2$ER compared to autologous red blood cell administration.\[^{27}\]

Several studies have been performed comparing hyperviscous fluids against their standard counterparts. In a hamster model of hemorrhagic shock, hyperviscous hetastarch administration resulted in higher functional capillary density compared to standard hetastarch
Another study compared the effects of low- and high-viscosity dextrans as well as a standard HBOC in extreme hemodilution. The high-viscosity dextran produced higher functional capillary density than the other fluids, as well as higher microvascular pressure across capillaries. The standard HBOC solution caused a rise in mean arterial pressure but decreased capillary perfusion pressure secondary to vasoconstriction. Measurement of perivascular nitric oxide concentration was performed in another study comparing hyperviscous plasma versus normal plasma transfusion in extreme hemodilution. The hyperviscous group had increased FCD as well as increased nitric oxide concentration. Wall shear stress was calculated in this experiment and correlated to the perivascular nitric oxide concentration. Additionally, administration of the hyperviscous plasma induced an increase in aortic endothelial nitric oxide synthase (one of the cNOS) activity. In all of these studies, maintenance of a plasma viscosity of approximately 2.1 cP was associated with beneficial microvascular effects.

To date, there is only one study evaluating the use of hyperviscous fluid resuscitation in a canine model of shock. In that study, dogs were randomized to receive standard LRS or hyperviscous LRS (created by addition of 0.5% alginate) following induction of severe hemorrhagic shock. In this study, the hyperviscous group demonstrated increased O2ER, decreased ScvO2, increased lactate, and required more fluid administration than the control group. The hyperviscous group did demonstrate significantly decreased vascular hindrance compared to the control group, consistent with vasodilation and potentially increased microvascular perfusion. Unfortunately, direct assessment of the microvasculature was not performed in this study.
Limited studies have been performed comparing hyperviscous HBOC solutions versus the normal counterparts. The results of one study indicated that maintaining physiologic viscosity during HBOC administration resulted in a delay in the onset of vasoconstriction (as determined by increased vascular hindrance) as well as a decrease in the magnitude of vasoconstriction.\textsuperscript{[98]} Administration of MP4, a naturally hyperviscous, hyperoncotic, low-p50 HBOC, has been associated with better survival in a rat model when compared to two hetastarch-based solutions. Another study, which compared MP4 to LRS, cell-free hemoglobin, and pentastarch in a porcine model of uncontrolled hemorrhagic shock also found improved survival with MP4.\textsuperscript{[99-100]} To date, there are no studies of hyperviscous HBOC administration in canine models of hemorrhagic shock.
Figure 1.1: Oxygen delivery curve

Figure 1.2: Compensatory responses to hemorrhagic shock
Figure 1.3: A microcirculatory unit

Figure 1.4: Schematic of the microcirculation
Figure 1.5: Nitric oxide-induced vasodilation

Figure 1.6: The Fahraeus-Lindqvist effect
Figure 1.7: Sidestream dark field microscopy

Figure 1.8: The MicroScan videomicroscope
Figure 1.9: Calculating the total vessel density on an SDF image
Figure 1.10: Calculating the MFI with AVA software

Figure 1.11: Structure of hemoglobin
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Class I</th>
<th>Class II</th>
<th>Class III</th>
<th>Class IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss (mL)</td>
<td>&lt;750</td>
<td>750-1500</td>
<td>1500-2000</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>Blood loss (%)</td>
<td>&lt;15%</td>
<td>15-30%</td>
<td>30-40%</td>
<td>&gt;40%</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>&lt;100</td>
<td>&gt;100</td>
<td>&gt;120</td>
<td>&gt;140</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Normal</td>
<td>Decreased</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>14-20</td>
<td>20-30</td>
<td>30-40</td>
<td>&gt;35</td>
</tr>
<tr>
<td>(breaths/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine output (ml/hr)</td>
<td>&gt;30</td>
<td>20-30</td>
<td>5-15</td>
<td>Negligible</td>
</tr>
<tr>
<td>CNS symptoms</td>
<td>Normal</td>
<td>Anxious</td>
<td>Confused</td>
<td>Lethargic</td>
</tr>
</tbody>
</table>

Table 1.1. Classification of hemorrhage. Modified from Gutierrez, et al. CNS= Central nervous system
Chapter 2: The experiment

2.1 Introduction

Regardless of inciting cause, hemorrhage results in a loss of vascular volume, red blood cells and oxygen-carrying capacity and, if left untreated or undiagnosed, can result in the development of hemorrhagic shock. Therapy for hemorrhagic shock involves fluid resuscitation to replace intravascular volumes and restore homeostasis. Despite years of research, there is no consensus on the ideal fluid therapy to achieve this goal. Numerous studies comparing crystalloids, colloids, and blood products have been performed, with varying results. Regardless of the fluid used, blood viscosity is decreased following hemorrhagic shock, owing to dilution from transcompartmental fluid shifting and the administration of hypoviscous fluids.

There are many determinants of microvascular perfusion, including blood viscosity. Viscosity induces production of nitric oxide secondary to increased shear stress on the endothelial surface, resulting in microvascular dilation the potential for increased oxygen and nutrient delivery to the tissues. During hemorrhagic shock, vasoconstriction results in decreased microvascular perfusion, and these derangements persist despite clinical resolution of shock. Several studies have evaluated treatment of hemorrhagic shock with hyperviscous fluid therapy, resulting in improvement in functional capillary density (FCD), a factor which is associated with...
increased tissue survival.\textsuperscript{[24-25, 29, 96, 102]} Traditionally, monitoring of resuscitation from hemorrhagic shock has focused on macrovascular parameters such as heart rate, mean arterial pressure, and indirect measurements of microvascular function such as ScvO2, lactate, and base excess.\textsuperscript{[30]} Until recently, direct imaging of the microcirculation has been limited to the laboratory setting, due to the cumbersome equipment needed. Sidestream dark field microscopy, a newer imaging technology, uses LED light to directly image red blood cells flowing through the microcirculation. The device is a handheld videomicroscope, making it practical for laboratory or clinical use. This device has been used extensively in human studies, as well as to a limited extent in dogs.\textsuperscript{[49-51, 56, 103-104]}

Hemoglobin-based oxygen carriers (HBOC) are modified purified hemoglobin solutions designed to restore oxygen carrying capacity following anemia or hemorrhage. While they are effective at carrying oxygen, there are several limitations to the commercially available HBOCs, the most important of which is severe vasoconstriction. Due to its small size, free hemoglobin is able to contact the endothelial surface, where it avidly scavenges nitric oxide.\textsuperscript{[84, 105-106]} In humans, this has been associated with an increased incidence of myocardial infarction and hypertension, and these side effects have prevented approval of these products for use in the U.S.\textsuperscript{[85-86]} Of additional concern, the p50 (partial pressure of oxygen at which 50\% of the hemoglobin is saturated) is much higher than physiological p50 (53 mmHg vs. 28 mmHg in the dog).\textsuperscript{[107]} This encourages
offloading of oxygen at the arteriolar level, and results in vasoconstriction through local regulation of blood flow. Several modified HBOCs have been developed in attempt to circumvent these problems, such as encapsulation in vesicles, and polymerization. However, none of these modifications have been successful in offsetting the nitric oxide scavenging. There is an HBOC approved for use in dogs (Oxyglobin®) which is an ultrapurified, gluteraldehyde polymerized bovine hemoglobin. This product has a mean viscosity of 1.37 cP, much less than the normal whole blood viscosity of the dog (5 cP). In our study, we evaluated whether enhancing the viscosity of Oxyglobin® would result in increased wall shear stress and promote enough nitric oxide production to offset the scavenging by the HBOC. Resuscitation with hyperviscous fluids has been performed in numerous studies, and these fluids restore microcirculatory flow (as characterized by intravital microscopy) better than their standard counterparts. Increased FCD is noted following hyperviscous resuscitation, and this finding is associated with increased tissue survival in hemorrhagic shock. We hypothesized that resuscitation with a hyperviscous HBOC would result in increased vasodilation compared to resuscitation with a standard HBOC. In addition, we hypothesized that resuscitation with both fluids would return macrovascular parameters to baseline, however, resuscitation with the hyperviscous fluid would also result in improved microcirculatory parameters, including perfused vessel density (analogous to functional capillary density).
2.2 Materials and Methods

All procedures were performed with approval from the Ohio State University Institutional Animal Care and Use Committee. Twelve conditioned male foxhounds, ranging from 1-6 years of age and with a mean 24.9 ± 2.56 kg (range, 19 to 29 kg) were fasted for 12 hours prior to procedures but were allowed free access to water. The sample size of twelve dogs was based on a power calculation seeking to find significant differences of at least 10% between treatment groups.

Preparation of the test fluid:

The control fluid was a standard HBOC (sHBOC) approved for use in dogs\textsuperscript{B}, with a mean viscosity of 1.37 cP. The test solution was made by adding alginate to the standard HBOC (hyperHBOC), resulting in a 0.3% solution. The solution was thoroughly mixed by use of a magnetic heated mixer. The resulting mean viscosity of the hyperHBOC was 4.96 cP.

Anesthesia and Instrumentation:

Each dog was instrumented with an intravenous catheter in the left cephalic vein, and the dogs were given hydromorphone\textsuperscript{C} (0.1 mg/Kg IV) for premedication. General anesthesia was induced with diazepam\textsuperscript{D} (0.5 mg/Kg IV) and ketamine\textsuperscript{E} (5-10 mg/Kg IV). The dogs were orotracheally intubated and maintained on sevoflurane gas\textsuperscript{F} administered in 100% oxygen. Mechanical ventilation was performed in order to maintain end-tidal carbon dioxide level of
35-45 mmHg. The dogs received fentanyl\(^G\) as a continuous rate infusion (6-10 mcg/Kg/hr) for continued analgesia and sedation. Lactated Ringer’s solution (LRS)\(^H\) was administered IV to all dogs through the duration of the procedure at a rate of 5 mL/Kg/hr. Continuous monitoring of heart rate, ETCO\(_2\), pulse oximetry\(^I\) (SpO\(_2\)), and electrocardiography\(^I\) was performed. A 20 gauge catheter was placed in the dorsal metatarsal artery for direct blood pressure measurement and lithium dilution cardiac output monitoring\(^K\). A 20 cm double lumen central venous catheter was placed in the left jugular vein for blood sampling, determination of central venous pressures and administration of lithium. The left carotid artery was surgically exposed for placement of a 10 French polyethylene catheter connected to a three-way stopcock. This catheter was used to obtain arterial blood samples and produce controlled hemorrhage. A splenectomy was performed through a ventral celiotomy incision in order to limit the variable hemodynamic effects of splenic contraction which can occur during hemorrhagic shock\(^{108-109}\). Electrocautery was used to promote hemostasis. The linea alba was closed to prevent dehydration and loss of heat, however a loop of jejunum was exposed and packed off in saline-soaked gauze for microvascular imaging. A mesenteric vein was catheterized for blood sampling. An 8-fr indwelling red rubber catheter was placed into the bladder for urine output monitoring.

**Experimental design:**

Following instrumentation and splenectomy, the dog was returned to right lateral recumbency and allowed a thirty minute equilibration period prior after
which baseline measurements were obtained. A stopcock and closed collection system containing heparin was connected to the carotid artery catheter and the dog was allowed to bleed freely until a MAP of 35-45 mmHg was achieved. The shock state was maintained for sixty minutes by removal of additional blood or administration of LRS (10 mL/Kg bolus) as needed. At the conclusion of the shock period, all data were collected.

The dogs were randomized by sealed envelope to either sHBOC or hyperHBOC for resuscitation. As suggested by the manufacturer, 30 mL/Kg of each solution was administered as a bolus over thirty minutes (administration of LRS was discontinued during this time). Resuscitation was considered to be achieved when a MAP of 65 mmHg was reached and sustained.

The dogs were maintained under general anesthesia, and all data was again collected at 30, 60, 120, and 180 minutes after resuscitation was complete. Following the final data collection, each dog was euthanized with 100 mg/Kg pentobarbital IV

**Measurement of macrohemodynamic, blood gas, and rheological variables**

Macrovascular variables measured included heart rate, central venous pressure (CVP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and MAP. Cardiac output was determined using the lithium dilution technique as previously described. Data obtained from arterial, mesenteric venous and central venous blood gas analysis included pH, pO₂, pCO₂, oxygen saturation (SO₂), lactate, base excess (BE), bicarbonate, total hemoglobin concentration, and
sodium. Sodium values were obtained for use in lithium dilution cardiac output determination.\textsuperscript{[110]} Packed cell volume (PCV) was determined by centrifugation and total plasma protein (TP) by refractometry. Colloid osmotic pressure (COP) was measured by a colloid osmometer\textsuperscript{N}. Whole blood viscosity and plasma viscosity were measured within 20 minutes after sampling on a DV-II+ Viscometer\textsuperscript{O} at a shear rate of 150 s\textsuperscript{-1} and temperature of 37°C. Calculated values included cardiac index (CI), systemic vascular resistance index (SVRI), systemic oxygen delivery (DO\textsubscript{2}), systemic oxygen consumption (VO\textsubscript{2}) and systemic oxygen extraction ratio (O\textsubscript{2}ER) using standard equations (Appendix B).

**Measurement of microvascular parameters**

At both time periods, videos of the buccal mucosal microcirculation were recorded using the videomicroscope.\textsuperscript{A} According to previously established consensus criteria, videos of 20 second duration are recommended.\textsuperscript{[28]} However, owing to technical and logistical limitations of the study design, videos of only 5 seconds in duration were obtained. For each dog at each time point, three videos were obtained by the same operator from adjacent areas of the buccal mucosa. In non-pigmented dogs, videos were obtained from the mucogingival junction above a carnassial tooth. In dogs with extensive oral pigmentation videos were obtained wherever non-pigmented mucosa or gingiva could be found. Care was taken to minimize application pressure and risk of vessel compression. The videos were then exported offline and analyzed using specialized vascular analysis software\textsuperscript{P}.
by a single investigator who was blinded as to the study time point at which the videos were obtained.

Videos were assessed for overall quality. Videos of insufficient quality (poor image resolution, excessive motion, pressure artifact, etc.) were withdrawn from analysis. The analysis software was then used to determine microcirculatory parameters established by consensus criteria.[28] Total vessel density (TVD) was derived by superimposing a grid of three equidistant horizontal and three vertical lines over the image. The TVD was then calculated based on the number of vessels crossing these lines divided by the length of those lines.[28] The proportion of perfused vessels (PPV) is a value based on the assignment of a flow category to each vessel (continuous, intermittent, or no-flow). The PPV was then calculated as $100 \times \left( \frac{\text{total number of vessels} - \left[ \text{no flow} + \text{intermittent flow} \right]}{\text{total number of vessels}} \right)$. The PVD was calculated by multiplying TVD and PPV. The analysis software was also used to determine the MFI. This value is obtained by dividing the visual field into quadrants and characterizing the flow as absent (0), intermittent (1), sluggish (2), or normal (3).[28] The value from each quadrant is then averaged to determine the MFI. Averaged measurements from videos of acceptable quality were used for determination of microcirculatory parameters for each subject at each timepoint.

In order to assess measurement reliability, intraobserver variability was determined for all microvascular parameters. Videos from eight dogs were randomly selected and analyzed in triplicate by the same investigator who
performed the study analysis. The investigator was blinded as to the previous video measurements as well as the hemodynamic state of the dog being analyzed. All statistics were performed with commercially available statistics software.\textsuperscript{QR}

**Statistical analysis:**

The data were analyzed for normality with the Kolmogorov-Smirnov test. To evaluate differences between groups and time points, a 2 way ANOVA for repeated measures was used. When significant differences were found, a Holm-Sidak post test was used to further characterize the differences between groups. For single-measure variables (such as time to resuscitation, cumulative urine output etc) a Student’s \textit{t}-test was performed to test for differences between control and experimental groups. For all tests, $p<0.05$ was considered statistically significant.

Intraobserver variability for microvascular parameters was determined with the coefficient of variation which is calculated by the equation: \textit{(standard deviation of the measurements/mean of measurements) X 100}.\textsuperscript{[112]}
2.3: Results

Macrovascular variables:

All data is presented as mean ± standard deviation. There were no significant differences between groups at baseline for any macrovascular variable. As expected, hemorrhagic shock resulted in tachycardia, metabolic acidosis, decreased $S_{cv}O_2$, and increased $O_2ER$ (figure 2.1-2.2, table 2.1-2.5) in all dogs with no significant differences between the groups. Following resuscitation, all macrovascular parameters returned to baseline (table 2.4) and blood and plasma viscosities were significantly higher in hyperHBOC dogs at all time points (fig 2.3-2.4). The hyperHBOC group had significantly higher SVRI, increased $O_2ER$, and decreased cardiac output and $S_{cv}O_2$ at several time points after resuscitation (table 2.4). There were no differences in mesenteric pO$_2$ between groups; however the hyperHBOC group had significantly lower mesenteric SO$_2$ only at the final timepoint. There were no other significant differences between groups for macrovascular variables.

For single comparison variables, there were no significant differences between groups for amount of blood removed to induce the shock state, time to resuscitation, HBOC administered until resuscitation, or total amount of crystalloid administered (table 2.6). Dogs in the sHBOC group produced significantly more urine over the course of the experiment.

Microvascular Variables:
No significant differences were noted at baseline or after shock between the hyperHBOC and sHBOC groups (Table 2.5). Perfused vessel density was significantly lower during hemorrhagic shock as compared to baseline. This was evident on gross inspection of the images as well as with determination of PVD with vascular analysis software. Microvascular flow index, TVD and PPV were also significantly decreased for both the buccal mucosa and jejunal serosa (Fig. 2.5-2.6). Following resuscitation, all microvascular parameters returned to baseline values and there were no significant differences between the groups. The coefficient of variation for intraobserver variability was within the accepted limits of <10% for all microvascular variables[112] (Table 2.7).
2.4: Discussion

As expected, induction of hemorrhagic shock resulted in profound hemodynamic derangements. While resuscitation with hyperHBOC resulted in increased plasma and blood viscosities compared to sHBOC, both fluids caused restoration of all macrovascular and microvascular variables to baseline, with no clinically relevant differences noted between the two groups.

During hemorrhagic shock, we found a significant reduction in all microvascular variables at both sites in all dogs. Decreased TVD indicates a decrease in the total number of capillaries seen on the video. The decrease in PPV indicates that a smaller proportion of the visible vessels had active flow compared to baseline, which may be a reflection of poor perfusion and heterogeneous distribution of blood flow. As a product of TVD and PPV, PVD decreased following shock, demonstrating both a decrease in vascular density as well as altered perfusion. The decrease in MFI could indicate a decrease in flow velocity, secondary to diminished blood volume and perfusion pressure, reflected as sluggish blood flow.

The mean 30 minute post-resuscitation plasma viscosity of the hyperHBOC group was 2.19 cP, similar to the ideal target viscosity described in other models of hyperviscous resuscitation.\(^{[66]}\) This elevated viscosity was sustained throughout the resuscitation period, as compared to the sHBOC group, which demonstrated a progressive decline in viscosity, likely secondary to transcompartmental fluid shifting as well as administration of hypoviscous fluid.
throughout the experiment. While a plasma viscosity of approximately 2.2 cP has been shown to be beneficial in murine models of severe hemodilution and hemorrhage,[26,66,90] it is possible that the degree of viscosity enhancement needed to effectively change microvascular perfusion is different in the dog.[66] In addition, a recent study used a mathematical model of endothelial-nitric oxide interactions to demonstrate that increasing wall shear stress up to 50 dynes/cm² was insufficient to overcome the effects of the nitric oxide scavenging by the HBOC fluid.[113] If the viscosity of the hyperHBOC dogs could not induce adequate nitric oxide production, that may also be a plausible explanation for the lack of significant microvascular changes in the hyperHBOC group compared to the sHBOC group.

Despite the apparent lack of benefit of increasing plasma viscosity on microvascular parameters in this study, it is important to note that all the microcirculatory parameters returned to baseline following resuscitation and were sustained at that level through the course of the monitoring period. As such, there was no microvascular evidence of vasoconstriction or resultant impairment of microcirculatory perfusion associated with administration of HBOC in either group of dogs. This is contrary to several published reports of vasoconstriction following administration of HBOCs.[105,114] This finding may relate to the severity of the shock model in this study, as loss of vasomotor tone is an key finding of fulminant, decompensated shock.[1] This occurs by two primary mechanisms; vascular hyporeactivity to vasoconstrictor molecules due to acidemia, as well as
massive endogenous nitric oxide production from the iNOS secondary to inflammation and severe global hypoxia.\textsuperscript{[14]} An additional consideration would be the use of inhalant anesthesia in this experiment. Volatile anesthetics such as sevoflurane have vasodilatory action on vascular smooth muscle cells and may blunt the response to catecholamines and other vasoconstrictive molecules.\textsuperscript{[115-116]} Further support for the vasodilatory effects of sevoflurane anesthesia may be reflected in the fact that there was not a significant change in SVRI during the shock period. A possible explanation for the lack of apparent vasoconstriction based on SVRI is that this value is a calculated macrovascular variable and may not directly reflect the microcirculation, especially if microvascular shunting or regional vasoconstriction is occurring. This theory is supported by the fact that evaluation of the microcirculatory parameters after shock suggests that vasoconstriction was occurring, despite the calculated SVRI.

Interestingly, the hyperHBOC group demonstrated increased SVRI compared to the sHBOC dogs at 120 and 180 minutes post resuscitation, although the value was not significantly different from baseline. If the hyperHBOC had induced vasodilation, the SVRI for the hyperHBOC group should have been lower at these time points when compared to the sHBOC group. At the same time points, cardiac index was significantly lower for the hyperHBOC dogs. This decrease in cardiac index could be due to increased resistance to flow secondary to the higher blood viscosity in these dogs. As SVRI is a calculated variable \([\frac{(\text{MAP}-\text{CVP})\times80}{\text{CI}}]\), the lower CI in the hyperHBOC dogs could cause an
elevation in calculated SVRI though it is not truly reflective of microvascular tone. This idea is substantiated by the fact that there was no evidence of significant reduction in microvascular parameters in the hyperHBOC group compared to baseline or the sHBOC group.

The hyperHBOC dogs demonstrated decreased $S_cO_2$ and $p_vO_2$, and increased $O_2ER$ at several time points compared with the sHBOC dogs. These findings are similar to another study which evaluated hyperviscous LRS resuscitation in canine hemorrhagic shock. Increased oxygen extraction may reflect poor resuscitation of the hyperHBOC group, as these variables are indirect indicators of tissue perfusion and oxygen consumption. Alternatively, if the hyperHBOC group had less microvascular shunting and better perfusion, these changes may represent repayment of the “oxygen debt” that was accumulated during the shock state. A recent study in septic humans demonstrated that patients with elevated $S_cO_2$ had higher mortality than people with normal $S_cO_2$, presumably due to microvascular shunting or mitochondrial dysfunction (dysoxia) in these patients.

There are several limitations to this study. A major limitation is that the dogs were maintained on sevoflurane gas and ventilated with 100% oxygen through the experiment. As discussed previously, inhalant anesthetics can directly influence vasomotor tone, and may have elevated microcirculatory parameters at baseline or obscured vasoconstriction secondary to hemorrhage or HBOC administration. Additionally, the dogs received ketamine as part of their
anesthetic induction. Ketamine has effects on the cardiovascular system, including increases in heart rate, myocardial work, cardiac output and myocardial oxygen demand. It has direct negative inotropic effects on the heart, as well as increased sympathetic outflow from the central nervous system. Therefore, the use of ketamine could have had an impact on microvascular perfusion. However, there was not a significant increase in heart rate at baseline associated with its administration and the expected decrease in PVD during hemorrhagic shock was still noted.

Our study also investigated hemorrhagic shock in a fixed-pressure, controlled hemorrhage model, with anesthetized dogs. This model generally has limited clinical applicability as the hemorrhage is controlled; hemostasis can be obtained immediately, allowing the investigator to titrate the shock severity to a predetermined endpoint. As hemorrhagic shock commonly occurs after severe trauma, the systemic effects of tissue trauma, inflammation and pain response can also have a significant impact. In fact, concurrent tissue injury and inflammation lowers the threshold for hemorrhage to induce shock. By performing laparotomy and splenectomy in these dogs we approximated the effects of tissue trauma and subsequent inflammatory response. However, due to humane concerns, we did not investigate the effects of pain or anxiety associated with trauma or hemorrhage, and this may have affected the results as well.

Another limitation of the study is that the microcirculatory videos were only five seconds in duration, rather than the recommended twenty seconds.
This diminished observation period was dictated by the effects of intestinal motility on the presence of motion artifact and its impact on video quality. A shorter video length may have led to overestimation of microvascular parameters if flow was intermittent over a full 20 seconds rather than the five seconds captured on the video. In addition, while all videos appeared to be of adequate technical quality at time of acquisition, during analysis several videos were deemed unacceptable secondary to excessive motion or pressure artifacts. Ultimately, 18 videos were removed from the analysis, which resulted in some dogs only having two videos available instead of the recommended three for analysis at certain time points.

There are limitations to use of SDF as well. In this study, we found that the MicroScan device works best on non-pigmented, non-keratinized tissue, and excessive salivation or blood in the video field is detrimental to video quality. In addition, acquisition of quality videos is an operator-dependent skill and there may a significant learning curve to this technology in dogs.\cite{50-51} The device must be in constant contact with the tissue in order to generate an image, however, excessive pressure may collapse the capillaries and lead to underestimation of microvascular parameters. One recommended way of assessing video quality involves evaluation for absence of flow in large veins. If this is noted, pressure artifact is likely present, and the pressure applied to the tissue should be reduced to provide a more accurate image of the microvasculature.\cite{28} Finally, the videos must be exported offline for analysis, and may take up to an hour to complete.
each video analysis. This may limit the clinical usefulness of the SDF technology.

**Conclusion:**

Based on the results of this study, shock resuscitation with a viscosity-enhanced HBOC solution does not appear to offer any advantages over the standard solution. Both study groups demonstrated return to baseline for all macro- and microvascular parameters, indicating successful resuscitation from shock. Direct imaging of the microcirculation indicated that vasoconstriction secondary to HBOC administration did not occur in our study. Dogs in the hyperHBOC group had increased O$_2$ER and decreased $S_{cv}O_2$ compared to the sHBOC dogs, which may indicate less microvascular shunting and decreased heterogeneity of microvascular flow, however this was not reflected by the microcirculatory parameters. While further investigation is warranted, based on the results of this study, enhancing the viscosity of HBOC solutions does not improve resuscitation and can not be recommended in the treatment of hemorrhagic shock in dogs.
Figure 2.1: Central venous oxygen saturation

Figure 2.2: Oxygen extraction ratio
Figure 2.3: Blood viscosity

Figure 2.4: Plasma viscosity
Figure 2.5: Perfused vessel density - buccal mucosa

Figure 2.6: Perfused vessel density - jejunal serosa
Figure 2.7: Microvascular Flow - buccal mucosa

Figure 2.8 Microvascular Flow - jejunal serosa
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Baseline</th>
<th>Shock</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>sHBOC</td>
<td>7.327±0.01</td>
<td>7.11 ±0.02</td>
<td>7.26 ±0.02</td>
<td>7.270 ±0.01</td>
<td>7.28 ±0.01</td>
<td>7.271 ±0.01</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>7.333±0.01</td>
<td>7.10 ±0.02</td>
<td>7.25 ±0.01</td>
<td>7.25 ±0.02</td>
<td>7.25 ±0.03</td>
<td>7.256 ±0.02</td>
</tr>
<tr>
<td>pCO₂ (mm/Hg)</td>
<td>sHBOC</td>
<td>45.55±0.97</td>
<td>68.02 ±1.17</td>
<td>71.17 ±2.34</td>
<td>53.09 ±1.79</td>
<td>52.63 ±1.59</td>
<td>52.43 ±1.34</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>45.47±0.91</td>
<td>74.27 ±2.13</td>
<td>74.02 ±1.78</td>
<td>53.67 ±3.08</td>
<td>56.08 ±3.61</td>
<td>54.60 ±2.90</td>
</tr>
<tr>
<td>pO₂ (mm/Hg)</td>
<td>sHBOC</td>
<td>83.55±10.9</td>
<td>39.20 ±3.29</td>
<td>78.26 ±2.36</td>
<td>77.01 ±9.43</td>
<td>80.37 ±7.93</td>
<td>80.83 ±7.8</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>83.95±7.26</td>
<td>36.20 ±2.02</td>
<td>71.96 ±3.89*</td>
<td>67.96 ±8.24*</td>
<td>56.72 ±4.9*</td>
<td>52.92 ±6.94*</td>
</tr>
<tr>
<td>SO₂ (%)</td>
<td>sHBOC</td>
<td>95.33±1.64</td>
<td>35.25 ±2.56</td>
<td>79.45 ±3.25</td>
<td>80.20 ±3.76</td>
<td>77.53 ±3.76</td>
<td>78.65 ±2.95</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>94.37±1.34</td>
<td>33.70 ±3.24</td>
<td>72.04 ±5.4*</td>
<td>70.61 ±1.75*</td>
<td>69.43 ±4.85*</td>
<td>67.05 ±5.10*</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>sHBOC</td>
<td>1.57±0.13</td>
<td>3.57±0.36</td>
<td>3.01±0.45</td>
<td>2.56±0.21</td>
<td>2.10±0.32</td>
<td>1.66±0.34</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>1.63±0.14</td>
<td>4.18±0.46</td>
<td>2.78±0.19</td>
<td>2.05±0.43</td>
<td>1.32±0.17</td>
<td>2.60±0.42</td>
</tr>
<tr>
<td>Base Exc. (mmol/L)</td>
<td>sHBOC</td>
<td>-1.92±0.54</td>
<td>-7.33 ±0.93</td>
<td>-3.34 ±0.39</td>
<td>-2.29 ±0.23</td>
<td>-2.03 ±0.26</td>
<td>-2.57 ±0.47</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>-1.63±0.74</td>
<td>-6.38 ±0.83</td>
<td>-3.65 ±0.56</td>
<td>-2.31 ±1.12</td>
<td>-2.37 ±1.95</td>
<td>-2.75 ±2.0</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>sHBOC</td>
<td>22.34±0.56</td>
<td>16.03 ±0.68</td>
<td>17.14 ±0.48</td>
<td>20.98 ±0.21</td>
<td>21.77 ±0.32</td>
<td>21.35 ±0.43</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>22.55±0.65</td>
<td>16.32 ±0.65</td>
<td>17.09 ±0.78</td>
<td>19.78 ±1.06</td>
<td>21.27 ±1.08</td>
<td>21.03 ±1.03</td>
</tr>
</tbody>
</table>

Table 2.1: Central venous blood gas parameters, *p<0.05
pO₂=partial pressure of oxygen; pCO₂= partial pressure of carbon dioxide; SO₂= oxygen saturation of hemoglobin; HCO₃⁻ = bicarbonate
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Baseline</th>
<th>Shock</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>sHBOC</td>
<td>7.36 ±0.04</td>
<td>7.20 ±0.04</td>
<td>7.24 ±0.07</td>
<td>7.32 ±0.05</td>
<td>7.34 ±0.03</td>
<td>7.34 ±0.01</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>7.37 ±0.03</td>
<td>7.18 ±0.04</td>
<td>7.25 ±0.07</td>
<td>7.34 ±0.05</td>
<td>7.34 ±0.06</td>
<td>7.34 ±0.02</td>
</tr>
<tr>
<td>pCO₂ (mm/Hg)</td>
<td>sHBOC</td>
<td>39.15 ±2.53</td>
<td>39.95 ±2.53</td>
<td>43.30 ±4.35</td>
<td>48.28 ±4.70</td>
<td>43.48 ±5.38</td>
<td>42.47 ±1.03</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>39.95 ±1.73</td>
<td>39.15 ±1.73</td>
<td>47.18 ±4.01</td>
<td>47.47 ±7.01</td>
<td>40.45 ±6.07</td>
<td>41.10 ±3.49</td>
</tr>
<tr>
<td>pO₂ (mm/Hg)</td>
<td>sHBOC</td>
<td>481 ±139</td>
<td>449 ±148</td>
<td>461 ±158</td>
<td>438 ±151</td>
<td>450.67 ±153</td>
<td>437 ±150</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>478 ±170</td>
<td>455 ±129</td>
<td>471 ±136</td>
<td>457 ±127</td>
<td>477.17 ±139</td>
<td>444 ±127</td>
</tr>
<tr>
<td>SO₂ (%)</td>
<td>sHBOC</td>
<td>100 ±0.02</td>
<td>100 ±0.00</td>
<td>99.72 ±0.14</td>
<td>100 ±0.00</td>
<td>99.13 ±0.51</td>
<td>99.38 ±0.03</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>100 ±0.00</td>
<td>100 ±0.00</td>
<td>100 ±0.07</td>
<td>100 ±0.00</td>
<td>98.99 ±0.93</td>
<td>99.10 ±0.09</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>sHBOC</td>
<td>1.73 ±0.36</td>
<td>4.20 ±1.18</td>
<td>2.92 ±1.29</td>
<td>1.83 ±0.76</td>
<td>1.27 ±0.82</td>
<td>1.45 ±0.59</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>1.80 ±0.36</td>
<td>4.62 ±1.22</td>
<td>3.52 ±1.80</td>
<td>2.50 ±1.26</td>
<td>2.25 ±0.82</td>
<td>2.43 ±0.99</td>
</tr>
<tr>
<td>Base Exc. (mmol/L)</td>
<td>sHBOC</td>
<td>-3.12 ±1.23</td>
<td>-10.31 ±2.43</td>
<td>-6.05 ±2.99</td>
<td>-3.25 ±1.90</td>
<td>-2.14 ±1.05</td>
<td>-2.63 ±1.21</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>-2.28 ±2.17</td>
<td>-10.13 ±1.99</td>
<td>-5.98 ±3.322</td>
<td>-3.57 ±2.95</td>
<td>-2.98 ±2.86</td>
<td>-3.48 ±2.54</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>sHBOC</td>
<td>Not available</td>
<td>Not available</td>
<td>18.93 ±2.60</td>
<td>21.5 ±1.64</td>
<td>22.25 ±0.93</td>
<td>22.1 ±1.00</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>Not available</td>
<td>Not available</td>
<td>19.10 ±2.61</td>
<td>21.38 ±2.36</td>
<td>21.87 ±2.43</td>
<td>21.47 ±2.19</td>
</tr>
</tbody>
</table>

Table 2.2: Carotid arterial blood gas parameters, *p<0.05
pO₂=partial pressure of oxygen; pCO₂= partial pressure of carbon dioxide; SO₂= oxygen saturation of hemoglobin; HCO₃⁻= bicarbonate
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Baseline</th>
<th>Shock</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>sHBOC</td>
<td>7.33 ±0.03</td>
<td>7.04 ±0.07</td>
<td>7.16 ±0.07</td>
<td>7.25 ±0.05</td>
<td>7.29 ±0.03</td>
<td>7.28 ±0.02</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>7.34 ±0.03</td>
<td>7.07 ±0.03</td>
<td>7.19 ±0.05</td>
<td>7.24 ±0.06</td>
<td>7.25 ±0.08</td>
<td>7.24 ±0.07</td>
</tr>
<tr>
<td>pCO₂ (mm/Hg)</td>
<td>sHBOC</td>
<td>46.08 ±3.06</td>
<td>80.3 ±8.16</td>
<td>59.47 ±3.82</td>
<td>55.67 ±4.61</td>
<td>52.23 ±3.12</td>
<td>52.25 ±2.42</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>45.54 ±2.46</td>
<td>77.22 ±10.01</td>
<td>63.67 ±8.44</td>
<td>54.97 ±8.42</td>
<td>57.02 ±9.26</td>
<td>56.22 ±9.16</td>
</tr>
<tr>
<td>pO₂ (mm/Hg)</td>
<td>sHBOC</td>
<td>72.5 ±24.17</td>
<td>42.27 ±8.03</td>
<td>84.11 ±22.68</td>
<td>73.13 ±17.54</td>
<td>73.77 ±15.80</td>
<td>77.45 ±18.44</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>67.63 ±15.41</td>
<td>47.77 ±7.01</td>
<td>74.88 ±17.25</td>
<td>59.07 ±9.91</td>
<td>55.48 ±12.80</td>
<td>52.31 ±11.58*</td>
</tr>
<tr>
<td>SO₂ (%)</td>
<td>sHBOC</td>
<td>90.03 ±6.52</td>
<td>34.53 ±9.02</td>
<td>80.56 ±3.23</td>
<td>74.80 ±2.86</td>
<td>78.60 ±7.63</td>
<td>80.67 ±5.66</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>90.73 ±5.00</td>
<td>42.73 ±7.49</td>
<td>79.15 ±6.15</td>
<td>77.23 ±4.18</td>
<td>72.60 ±5.98</td>
<td>70.85 ±5.90*</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>sHBOC</td>
<td>1.68 ±0.50</td>
<td>4.85 ±1.44</td>
<td>3.20 ±1.38</td>
<td>2.15 ±0.98</td>
<td>1.32 ±0.43</td>
<td>1.58 ±0.61</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>1.52 ±0.25</td>
<td>4.80 ±0.87</td>
<td>4.02 ±1.79</td>
<td>3.18 ±1.65</td>
<td>2.65 ±1.23</td>
<td>2.81 ±1.21</td>
</tr>
<tr>
<td>Base Exc. (mmol/L)</td>
<td>sHBOC</td>
<td>-1.25 ±1.70</td>
<td>-8.52 ±3.05</td>
<td>-5.41 ±3.23</td>
<td>-2.50 ±2.10</td>
<td>-1.50 ±1.09</td>
<td>-2.02 ±1.25</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>-0.62 ±2.33</td>
<td>-8.02 ±1.98</td>
<td>-5.40 ±3.38</td>
<td>-3.50 ±2.88</td>
<td>-2.48 ±2.81</td>
<td>-3.17 ±2.86</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>sHBOC</td>
<td>22.66 ±1.85</td>
<td>14.73 ±2.32</td>
<td>18.70 ±2.74</td>
<td>21.23 ±1.83</td>
<td>22.23 ±2.59</td>
<td>21.85 ±1.01</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>23.25 ±1.27</td>
<td>15.12 ±1.46</td>
<td>18.53 ±2.43</td>
<td>20.40 ±2.32</td>
<td>21.13 ±1.10</td>
<td>20.63 ±2.50</td>
</tr>
</tbody>
</table>

Table 2.3: Mesenteric venous blood gas parameters, *p<0.05

pO₂=partial pressure of oxygen; pCO₂= partial pressure of carbon dioxide; SO₂= oxygen saturation of hemoglobin; HCO₃⁻= bicarbonate
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Baseline</th>
<th>Shock</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>sHBOC</td>
<td>71.8 ±14.8</td>
<td>210 ±9.14</td>
<td>91.2 ±18.0</td>
<td>104 ±22</td>
<td>95.5 ±19</td>
<td>101 ±20.5</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>54.0 ±5.14</td>
<td>200.3 ±6.35</td>
<td>96.0 ±21.3</td>
<td>98 ±11.67</td>
<td>115.2 ±15.2</td>
<td>125 ±19.0</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>sHBOC</td>
<td>136.8 ±9.7</td>
<td>43 ±1.86</td>
<td>144.5 ±10.9</td>
<td>151 ±4.56</td>
<td>144.0 ±12.2</td>
<td>142.0 ±8.8</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>147.5 ±8.4</td>
<td>46.2 ±2.1</td>
<td>142.3 ±15.2</td>
<td>147 ±7.89</td>
<td>150.3 ±9.79</td>
<td>158.3 ±15.3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>sHBOC</td>
<td>91.33 ±8.1</td>
<td>37.67 ±1.3</td>
<td>92.23 ±9.14</td>
<td>86.79 ±2.35</td>
<td>90.50 ±8.79</td>
<td>89.83 ±4.95</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>96.83 ±7.6</td>
<td>39.00 ±0.58</td>
<td>93.48 ±11.2</td>
<td>92 ±4.47</td>
<td>100.5 ±6.44</td>
<td>110.8 ±9.96*</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>sHBOC</td>
<td>78.83 ±8.02</td>
<td>32.67 ±1.1</td>
<td>81.23 ±5.8</td>
<td>79.36 ±7.23</td>
<td>73.33 ±6.33</td>
<td>85.17 ±16.7</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>81.17 ±6.5</td>
<td>33.67 ±0.56</td>
<td>83.46 ±6.65</td>
<td>81.87 ±10.2</td>
<td>86.33 ±5.92</td>
<td>93.17 ±10.1</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>sHBOC</td>
<td>3.53 ±0.37</td>
<td>1.51 ±0.17</td>
<td>3.33 ±0.21</td>
<td>3.38 ±0.12</td>
<td>3.04 ±0.19</td>
<td>3.39 ±0.15</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>2.96 ±0.28</td>
<td>1.41 ±0.34</td>
<td>2.24 ±0.18*</td>
<td>2.34 ±0.31</td>
<td>2.21 ±0.27*</td>
<td>2.56 ±0.20*</td>
</tr>
<tr>
<td>SVRI (dyne*sec/cm⁵)</td>
<td>sHBOC</td>
<td>2143 ±421</td>
<td>2175 ±57</td>
<td>2100 ±302</td>
<td>2439 ±238</td>
<td>2338 ±261</td>
<td>2067 ±129</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>2608 ±334</td>
<td>2797 ±501</td>
<td>2068 ±459</td>
<td>3441 ±573</td>
<td>3361 ±293*</td>
<td>3402 ±598*</td>
</tr>
<tr>
<td>VH</td>
<td>sHBOC</td>
<td>544 ±314</td>
<td>802 ±322</td>
<td>733 ±193</td>
<td>1045 ±172</td>
<td>1219 ±291</td>
<td>1094 ±202</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>648 ±228</td>
<td>1000 ±462</td>
<td>806 ±183</td>
<td>787 ±119</td>
<td>1158 ±362</td>
<td>1156 ±187</td>
</tr>
</tbody>
</table>

Table 2.4: Macrovascular variables

* p<0.05
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Baseline</th>
<th>Shock</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVD-BM (mm/m²)</td>
<td>sHBOC</td>
<td>13.62 ±2.6</td>
<td>10.40 ±4.4</td>
<td>13.28 ±2.6</td>
<td>12.93 ±3.0</td>
<td>13.06 ±2.4</td>
<td>12.18 ±3.1</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>14.53 ±2.3</td>
<td>10.94 ±3.4</td>
<td>13.43 ±2.4</td>
<td>13.03 ±2.3</td>
<td>11.34 ±2.5</td>
<td>13.48 ±2.0</td>
</tr>
<tr>
<td>TVD-JS (mm/m²)</td>
<td>sHBOC</td>
<td>13.14 ±1.8</td>
<td>9.67 ±2.3</td>
<td>12.69 ±1.8</td>
<td>11.62 ±2.1</td>
<td>11.65 ±2.0</td>
<td>11.88 ±2.2</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>10.00 ±2.7</td>
<td>9.28 ±3.0</td>
<td>10.60 ±2.6</td>
<td>12.10 ±2.1</td>
<td>12.17 ±3.7</td>
<td>11.15 ±2.4</td>
</tr>
<tr>
<td>PVD-BM (mm/m²)</td>
<td>sHBOC</td>
<td>14.43 ±2.7</td>
<td>6.76 ±3.7</td>
<td>13.58 ±2.8</td>
<td>12.84 ±3.9</td>
<td>13.4 ±2.9</td>
<td>12.75 ±5.3</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>14.80 ±3.0</td>
<td>6.88 ±5.8</td>
<td>12.00 ±5.5</td>
<td>10.74 ±4.0</td>
<td>10.3 ±3.2</td>
<td>13.04 ±2.6</td>
</tr>
<tr>
<td>PVD-JS (mm/m²)</td>
<td>sHBOC</td>
<td>12.70 ±2.8</td>
<td>5.21 ±2.5</td>
<td>10.47 ±4.7</td>
<td>8.42 ±3.0</td>
<td>8.78 ±2.9</td>
<td>7.55 ±3.5</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>9.80 ±2.5</td>
<td>4.80 ±3.6</td>
<td>9.32 ±5.0</td>
<td>11.10 ±2.6</td>
<td>11.12 ±3.2</td>
<td>11.05 ±2.9</td>
</tr>
<tr>
<td>PPV-BM (%)</td>
<td>sHBOC</td>
<td>81.88 ±8.3</td>
<td>63.19 ±26</td>
<td>81.74 ±12</td>
<td>78.32 ±17</td>
<td>84.07 ±9</td>
<td>80.83 ±28</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>83.64 ±9.0</td>
<td>51.23 ±33</td>
<td>70.41 ±34</td>
<td>68.77 ±22</td>
<td>77.1 ±20</td>
<td>87.45 ±10</td>
</tr>
<tr>
<td>PPV-JS (%)</td>
<td>sHBOC</td>
<td>84.35 ±7.5</td>
<td>39.24 ±20</td>
<td>77.38 ±31</td>
<td>67.0 ±16</td>
<td>70.50 ±20</td>
<td>60.61 ±28</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>89.12 ±6.7</td>
<td>42.74 ±30</td>
<td>79.50 ±31</td>
<td>84.7 ±11</td>
<td>74.12 ±23</td>
<td>91.03 ±7.3</td>
</tr>
<tr>
<td>MFI-BM</td>
<td>sHBOC</td>
<td>2.53 ±0.43</td>
<td>1.71 ±0.78</td>
<td>2.60 ±0.32</td>
<td>2.38 ±0.73</td>
<td>2.50 ±0.47</td>
<td>2.47 ±0.98</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>2.63 ±0.47</td>
<td>1.43 ±0.92</td>
<td>2.31 ±0.95</td>
<td>2.16 ±0.82</td>
<td>2.39 ±0.55</td>
<td>2.34 ±0.51</td>
</tr>
<tr>
<td>MFI-JS</td>
<td>sHBOC</td>
<td>2.61 ±0.32</td>
<td>1.05 ±0.73</td>
<td>2.29 ±0.70</td>
<td>2.06 ±0.73</td>
<td>2.20 ±0.51</td>
<td>1.92 ±0.58</td>
</tr>
<tr>
<td></td>
<td>HyperHBOC</td>
<td>2.65 ±0.36</td>
<td>1.11 ±0.80</td>
<td>2.38 ±0.74</td>
<td>2.41 ±0.37</td>
<td>2.42 ±0.56</td>
<td>2.57 ±0.56</td>
</tr>
</tbody>
</table>

Table 2.5 Microvascular variables *p<0.05
TVD-BM= total vessel density (buccal mucosa); TVD-JS= total vessel density (jejunal serosa) PVD-BM=perfused vessel density (buccal mucosa); PVD-JS=perfused vessel density (jejunal serosa); PPV-BM= proportion of perfused vessels (buccal mucosa); PPV-JS=proportion of perfused vessels (jejunal serosa); MFI-BM=microvascular flow index (buccal mucosa); MFI-JS=microvascular flow index (jejunal serosa)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>sHBOC</th>
<th>HyperHBOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Removed mL/Kg</td>
<td>52.69 ± 9.3</td>
<td>50.49 ± 5.4</td>
</tr>
<tr>
<td>Urine Output mL/Kg</td>
<td>122 ± 32*</td>
<td>81 ± 21*</td>
</tr>
<tr>
<td>Time to Resuscitation (min)</td>
<td>4.62 ± 0.8</td>
<td>6.62 ± 2.1</td>
</tr>
<tr>
<td>HBOC to Resuscitation (mL)</td>
<td>118 ± 23</td>
<td>145 ± 65.5</td>
</tr>
<tr>
<td>Total LRS mL/Kg</td>
<td>33.3 ± 7.4</td>
<td>35.6 ± 8.1</td>
</tr>
</tbody>
</table>

**Table 2.6: Single-comparison variables**  
*p=0.03

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVD</td>
<td>3.87</td>
</tr>
<tr>
<td>PVD</td>
<td>3.87</td>
</tr>
<tr>
<td>PPV</td>
<td>3.26</td>
</tr>
<tr>
<td>MFI</td>
<td>3.74</td>
</tr>
</tbody>
</table>

**Table 2.7: Intraobserver variability for video analysis. Acceptable level <10%**

TVD= total vessel density; PVD=perfused vessel density; PPV=proportion of perfused vessels; MFI= microvascular flow index
Bibliography


### Appendix A: List of proprietary materials

A Microscan – MicroVision Medical, Amsterdam, The Netherlands

B Oxyglobin® – Biopure Inc.,

C Hydromorphone – Baxter Healthcare, Deerfield, IL

D Diazepam – Hospira Inc., Lake Forest, IL

E Ketamine (Ketaset®) – Fort Dodge Animal Health, Fort Dodge, IA

F Sevoflurane (SevoFlo®) – Abbott Animal Health, Abbott Park, IL

G Fentanyl citrate – Hospira Inc, Lake Forest, IL

H Lactated Ringer’s Solution – Baxter Healthcare, Deerfield, IL

I Passport 2®, Datascope Corp., Fairfield, NJ

J Datex, Instrumentarium Corp., Helsinki, Finland

K LiDCO – LiDCO Ltd, London, UK

L Euthanasia solution (Euthasol™) – Virbac Animal Health, Fort Worth, TX

M Blood gas – ABL 725, Radiometer America, Westlake, OH

N Colloid osmometer – model 4420, Wescor, Logan, UT

O Viscometer – Brookfield Engineering Laboratories, Middleboro, MA

P AVA 3.0 MicroScan Analysis Software, MicroVision Medical, Amsterdam, The Netherlands
PASW Statistics 18 (formerly SPSS Statistics), Chicago, IL

SigmaStat 3.5©; Systat, San Jose, CA
Appendix B: List of common cardiovascular equations

Oxygen content:
\[ C_{a}O_2 = (1.34 \times Hb \times SO_2) + 0.003p_aO_2 \text{ (mLO}_2/dL \text{ blood)} \]

Oxygen delivery:
\[ DO_2 = CI \times C_{a}O_2 \text{ (mL/min)} \]

Oxygen Consumption:
\[ VO_2 = CI \times (C_{a}O_2 - C_{v}O_2) \text{ (mL/min)} \]

Oxygen extraction ratio:
\[ O_2ER = (C_{a}O_2 - C_{v}O_2) / C_{a}O_2 \% \]

Systemic vascular resistance index:
\[ SVRI = [(MAP-CVP) \times 80] / CI \text{ (dynes/sec/cm}^5) \]

Vascular hindrance
\[ VH = SVRI / \text{viscosity} \]