Examination of the canine sublingual and gastric microcirculation in health and gastric dilatation volvulus

THESIS

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Abstract

The microcirculation consists of arterioles, capillaries, and venules measuring <200 μm and are responsible for delivery of nutrients, such as oxygen and glucose, to tissues and removal of waste products, such as carbon dioxide and lactate. In health, the flow within these vessels is maintained locally through a process called autoregulation. However, when diseases develop, this autoregulation may be lost. Commonly monitored macrohemodynamics, such as heart rate and blood pressure, have been evaluated and in general have not been found to correlate well with microvascular perfusion. Therefore, direct examination of the microcirculation has become an area of exploration in both the research and clinical settings. One such tool is sidestream dark field microscopy (SDM), which has utilized in both human and veterinary medicine.

The microcirculation has been studied in a variety of tissue beds and in several disease conditions. In humans, several studies have found a correlation between the sublingual tissue and the gastric tissue, making the sublingual site a feasible surrogate for assessing gastrointestinal perfusion. However, few studies have been performed in veterinary medicine. Those previously published studies have evaluated the buccal mucosa and intestinal mucosa; only one other study has evaluated the sublingual mucosa. No studies have evaluated microcirculation in gastric dilatation volvulus, even though this disease is relatively prevalent in the veterinary population and is likely to result in
both local and systemic microcirculatory derangement. Further, no veterinary studies have attempted correlation between the sublingual mucosa and the gastric serosa. These studies were designed to help explore this area further. Our first hypothesis was that intra-operative evaluation of sublingual and gastric tissue microcirculation would be feasible. Additional hypotheses were that there would be correlation between microvascular variables for these tissue beds, but there would be a lack of correlation with measured systemic variable. We also hypothesized that the microcirculation would be altered in GDV patients compared to a control population, but there would be improvement after gastric derotation.

The first study performed by our group evaluated normal anaesthetized dogs receiving a prophylactic gastropexy. Using SDM, we evaluated the sublingual mucosal and gastric serosal microcirculation and concurrent systemic variables. Microcirculatory parameters were compared and evaluated for correlation between the tissue beds. Additionally, we evaluated for correlation between the microcirculation variables and systemic variables. The second study evaluated microcirculatory and systemic variables in patients with naturally occurring GDV, at several time points. Using SDM, we evaluated the sublingual mucosal and gastric serosal microcirculation at induction, prederotation, postderotation and on recovery. The microcirculation was evaluated for the number of vessels present and the perfusion of these vessels, including how many are perfused and the quality of the blood flow. Each of these features was then compared to previously established control dog variables and between the time points. The systemic
variables were also compared to the microcirculation variables for evaluation of correlation.

These studies successfully demonstrated the ability to perform intraoperative assessment of gastric and sublingual microcirculation. However, there did not appear to be significant correlation between the tissue beds in either patient population, making the sublingual tissue a poor surrogate for gastric tissue. Although a few correlations were found between the microvascular parameters and systemic variables, these are unlikely to be clinically significant. Finally, when compared to the microvascular variables of normal dogs, those dogs with naturally occurring GDV had altered variables at all time points. Furthermore, although gastric derotation resulted in significant improvement in gastric microcirculation, these values failed to return to those seen in the control population. Overall, we established the utility of SDM technology for evaluation of canine sublingual and gastric tissues. These results may be useful for future studies of normal canine abdominal organs as well as in disease states.
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Chapter 1: Introduction

1.1. The vasculature: An Overview

The blood supply within the mammalian body has been extensively studied by physicians and scientists for centuries. Although a large portion of the vascular structure and function has been elucidated, mysteries remain which allow for the continued examination of this fascinating body system. Within the grown mammal, the cardiovascular system is one of the largest systems in the body, and is often divided into the pulmonic and systemic circulation. The systemic circulation at any one point contains approximately 84% of the entire blood volume; of this volume 64% is within the veins, 13% within the arteries and 7% within the arterioles and capillaries.1 Given the functional and structural complexity of this system, there can be further division of vasculature into the macrocirculation and microcirculation.

1.2 Vascular anatomy

1.2.1 Macrocirculation

The macrocirculation consists of all vessels measuring greater than 100 μm to 300 μm in diameter.2,3 These vessels have unique properties described as viscoelastic, or having both an elastic and a viscous nature.4 This allows for deformation of the vessels which is determined by the stress that is placed upon it but ensures that it will return to its original state after the stress is removed. These are properties which are essential for
large blood vessels to propagate blood flow downstream. This feature is enabled by the elastic materials which largely comprise the arterial wall, primarily elastin and collagen complexes with mucoprotein. Within great vessels, elastin dominates while progressively more distal vessels contain mostly collagen, especially in arterial circulation. This transitioning composition is echoed in the increasing stiffness of the vessels.

There are three layers that compose vessel wall structure; thickness and specific composition of the individual layers varies at different levels of the circulatory tree. The inner most layer is the tunica intima, consisting of the vascular endothelial cells and a thin layer of elastin and collagen fibers. The central layer is the tunica media containing a fibrous network between smooth muscle cells. These smooth muscle cells are arranged circumferentially within the elastin and collagen fibers and contribute to the modulation of wall tension, contraction and relaxation of the vessel. The final outer layer, tunica adventitia, is created from collagen and elastin fibers interconnecting with surrounding connective tissue.

Vascular innervation is contained within the tunica adventitia and contributes to variations of resting vascular tone. This innervation is found throughout the cardiovascular system, except for capillaries, and is primarily mediated by adrenergic input (although some cholinergic input may be present). Among the systems of the body, some have stronger systemic innervation while others have a balance of sympathetic and parasympathetic. Some examples include the gastrointestinal tract, pulmonary tree and the heart, which contain both sympathetic and parasympathetic innervation. In
comparison, vascular smooth muscle, kidneys and sweat glands primarily have sympathetic activity.\textsuperscript{5} This general layering of the vascular anatomy continues into the smaller vessels, although structural changes make distinct differences between the macrocirculatory and microcirculatory anatomy.

1.2.2 Microcirculation

The microcirculation encompasses all vessels with a diameter of less than 100 μm to 300 μm and includes approximately 99% of all vessels of the cardiovascular system.\textsuperscript{2,3} The overall vessel structure creates a mesh network embedded within the tissues of the body.\textsuperscript{6} These vessels have a heterogeneity of both path length and number of vessel segments, with the organization of the vessels dependent upon the respective tissue it is supplying.\textsuperscript{2,6} Although further description microcirculatory anatomy is provided later in this section, a brief difference between two vascular beds, the pulmonary and cerebral microcirculation, will be briefly described. The pulmonary capillaries often do not diverge from precapillaries but branch directly off arterioles larger than 100 μm.\textsuperscript{6} These arterioles may contain intermittent areas of smooth muscle, allowing for the vasoconstrictive response to regional hypoxia\textsuperscript{6}, a vital necessity for maintaining arterial oxygen levels. As the capillaries transverse along the tissue, some may run in parallel to the larger vessels, forming a periarterial network.\textsuperscript{6} Also contrasting other microcirculatory beds, pulmonary capillaries at baseline are normally fully dilated.\textsuperscript{6} In comparison, the cerebral microcirculation involves a large number of arterioles on the brain surface with numerous small arteriovenous anastamoses.\textsuperscript{6} Another important aspect of cerebral microcirculation is the small arterioles that branch and then penetrate
into the deep parenchymal tissue.\(^6\) This ensures perfusion to the essential functional parenchyma. The particular features of these two different tissue beds demonstrate how the microcirculation must adapt for functionality.

The three layers that compose the vascular walls are continued from the macrocirculation, though there is some variation with transition into the microcirculation. As such, there are several classifications that can be used to distinguish the varying levels of the microcirculation. One classification system is based upon topology: arterioles serve to connect diverging bifurcations, capillaries connect diverging and converging bifurcations and venules connect the converging bifurcations.\(^2\) Additional portions of the microcirculation are the metarterioles, which are the terminal arterioles before truncating into capillaries, and arteriovenous anastamoses that are short connections between arterial and venous microcirculation.\(^1,2\) Figure 1 below is a schematic picture of the vascular segments composing a microcirculatory unit. Smooth muscle found around the arterioles and at the precapillary sphincters.\(^7\)
Microcirculatory arterioles consist of a tight layer of endothelial cells surrounded by an internal elastic lamina and are highly muscular, created by multiple layers of vascular smooth muscle.\textsuperscript{1, 6} These arterioles branch in a relatively orderly manner before transitioning to metarterioles.\textsuperscript{6} This transition includes the loss of a continuous muscle layer, although smooth muscle fibers continue to intermittently encircle the vessels. Encircling muscle fibers are distinguished as precapillary sphincters which are important in blood flow regulation, to be discussed later.\textsuperscript{1} The metarterioles end at the capillary beds.

Capillaries consist of a single layer of endothelial cells surrounded by a basement membrane.\textsuperscript{1} The tightness of the endothelial cell connection is maintained by various protein complexes and is dependent upon the organ location of the capillary bed.\textsuperscript{1, 2} A prominent feature of capillaries is the complete lack of smooth muscle,\textsuperscript{1} making these vessels unable to regulate their own tone/diameter as can vessels higher in the vascular
tree. However, at irregular intervals, pericytes, a type of supportive cell, may surround the capillaries and may have contractile abilities. Overall, true capillaries have a very limited ability to affect flow and thus are dependent upon upstream and local regulation. Capillary vessels converge to create venules which eventually join with veins.

Venules consist of endothelial cells surrounded by a basement membrane and pericytes (early in the branch work). However there is no evidence of smooth muscle until they are larger than 30 μm in diameter, after which they will progressively have more smooth muscle and more innervation. The final component of microcirculation is the arteriovenous anastomoses, short and direct connections between the arterioles and venules. These vessels have been found to have more innervation in the adventitial layer than even arteries. Flow through these vessels may be intermittent and dependent upon the tissues’ functional requirements. They also serve as the potential conduit for microvascular shunting.

Though often schematized as consistent and symmetrical unit, the microvascular arcade actually has an irregular and random pathway of blood flow, as well as significant additional heterogeneity within the capillary diameters. Given the complexity of this system in regards to anatomy and variation within individual vessels, along with regional differences in regulation, data from a single vessel makes analysis of function and vascular flow challenging to interpret as an overall vascular bed. As such, more accurate assessment of tissue perfusion requires consideration of the entire local microcirculatory unit.
1.3 General blood flow determinants

A large number of factors determine or affect blood flow within the vasculature. Classically, many components are best considered with Poiseuille’s Law, which describes laminar flow through a rigid tube. Poiseuille’s Law is:

\[ Q = \frac{\pi}{8} \cdot \frac{r^4}{l} \cdot \frac{1}{\eta} \cdot \Delta P \]

Where \( Q \) represents flow, \( r \) is the radius of the vessel, \( l \) is the length of the vessel, \( \eta \) is the fluid property that is resistant to shearing motions (viscosity), and \( \Delta P \) is the change of pressure along the vessel. Additional consideration can be given to each portion to further explain the concept of blood flow for any vessel size. The radius has marked effect on the flow through the vessels, with a two-fold decrease in vessel radius causing a decrease in flow by a factor of sixteen. As will be discuss later, many factors will influence the vascular tone/size (vascular geometry) and therefore affect blood flow.

Flow is also inversely related to the length of the tube, with a shorter vessel having the potential for faster flow. The pressure change along the length of the vessel is the major driving force for flow and is dependent upon the resistance within the vessel. Finally, another significant determinant is the fluid property (viscosity) of the blood.

In normal circumstances, red blood cells have the largest influence on blood flow properties and on vascular shear rate. Shear rate is the rate at which two different layers of fluid move in respect to each other. As hematocrit increases, shear rate decreases leading to an increase in blood viscosity. However if shear rate also increases (even with increase of hematocrit), there will be a decrease in viscosity. This is likely from the impact that shear rate has on red blood cell aggregation. A natural aggregation
of red blood cells can occur within vessels called rouleaux. As there is an increase of the shear rate, the length or amount of rouleaux formed within the vessel decreases from the shearing forces.\textsuperscript{4} This has a dramatic impact on the viscosity of that blood.

Normal blood flow through a larger vessel has been described as a parabolic, with the central portion laminar (little to no flow interference) and the flow nearer the vessel wall as more turbulent.\textsuperscript{1,4} While some turbulence is expected, excessive turbulence can be detrimental. The likelihood or potential for turbulent flow can be assessed through determination of Reynolds number, which takes into account several factors and can be represented by the following equation:

\[
\text{(Eq. 2) } \text{Re} = \frac{(v \cdot d \cdot \rho)}{\eta} \text{\textsuperscript{1}}
\]

Where Re is Reynolds number, \(v\) is velocity of flow, \(d\) is diameter of the vessel, \(\rho\) is fluid density, and \(\eta\) is viscosity. The pulsatile nature of flow within the circulatory system further increases the potential for turbulence.\textsuperscript{4} While there is the natural tendency for turbulent flow, occasionally creating eddy currents (whorls of blood), the presence of turbulence leads to additional resistance to flow within the vessels and can lead to pathology.\textsuperscript{1,4} Turbulence can lead to red blood cell fragmentation, hemolysis, loss of red blood cell concavity, and the risk of thrombus formation.\textsuperscript{4} Risk of turbulence in branched blood vessels occurs when the Reynolds number is between 200 to 400 but becomes less turbulent in straight vessels.\textsuperscript{1} However, when the Reynolds number is over 2000, defined as the critical Reynolds number\textsuperscript{4}, even within the larger vessels, the flow has a greater tendency for turbulence.\textsuperscript{1} And if Reynold’s number exceeds 4000, continuous turbulent flow will almost always occur.\textsuperscript{8}
Arterial tapering and distensibility of the vessels are vascular characteristics aimed at minimizing turbulent flow. Features of optimal blood flow have been evaluated and take into account both the fluid itself and the vascular environment. The normal composition and viscosity of blood, including cellular and plasma components, should be present. This includes a homogeneous distribution of red blood cells, white blood cells and platelets which do not adhere to each other or the endothelium. Velocity of the flow in arterioles and venules should be streamlined with individual erythrocytes having no abnormal membrane attachments or conformational change. Finally, normal blood flow requires a well maintained vascular wall that is nourished and has a balanced permeability. Failure of one or more of these characteristics may substantially increase the risk of turbulence and its pathological consequences.

1.4 Blood flow within the microcirculation

While the general principles of blood flow persist within the microcirculation, there are some specific concepts to consider. Given vascular anatomy, vessel diameter may be less than eight micrometers. This is comparable to the size of a human red blood cells which results in marked reduction in the flow, as described above (Eq. 1). The flow of blood within the microcirculation may not be continuous, as seen in larger vessels, with red blood cells often in single file. At such small diameters, factors must be in place to ensure continued flow of blood within capillaries. Some of these factors include the response of endothelial cells to an increase in shear stress. This may allow for endothelial derived metabolites, such as nitric oxide, to vasodilate and allow continued flow. Another important factor is the relationship of a decreasing velocity as
vessel diameter decreases.\textsuperscript{6} This is known as the Fåhraeus-Lindqvist effect, which will be discussed later in this section.

As blood flow transitions from arterioles to capillaries, red blood cells and plasma may disproportionately distribute within branches.\textsuperscript{6} If the capillary beds are collapsed or precapillary sphincters prevent blood flow into capillaries, the metarterioles will function as a shunt for blood from the arterioles to venules. Within arterioles, shear stress prevents aggregation of red blood cells.\textsuperscript{6} If red blood cells have difficulty entering into the small branches, this is described as red blood cell skimming.\textsuperscript{6} Aggregation within capillary beds is often inhibited due to the narrow diameter.\textsuperscript{6} However, if aggregation were to occur with the microvascular network, its significance is difficult to predict. These aggregates can result in changes to red blood cell distribution\textsuperscript{6}, which may lead to significantly altered flow through capillaries or result in capillary plugging.

As discussed, blood flow through the microcirculation may also be affected by fluid characteristics. One important factor affecting microcirculatory blood flow is hematocrit. As discussed, the red blood cells have a significant effect on fluid viscosity. Within the microcirculation, there is a reduction of hematocrit within small vessels as red blood cells are forced into single file. This allows for an increase in fluid transit as the ratio of red blood cell to plasma diminishes. This is known as the Fåhraeus effect.\textsuperscript{6} In relation to the fluid viscosity, the viscosity declines with diameter of the vessel, which can be described as the Fåhraeus-Lindqvist effect,\textsuperscript{6} and which is depicted below in Figure 2.\textsuperscript{9} Finally, microcirculatory flow can be impacted by intravascular pressure. In a small number of vessel segments, there is a large pressure drop from high pressure and shear
stress to low pressure and low shear stress.\textsuperscript{6} This pressure drop is especially important within vessels that are in the functional exchange region of the microcirculation, allowing for improved nutrient exchange.\textsuperscript{6} Although intermittent blood flow within the microvascular system may be significantly affected by these vascular components, regulation of blood flow also contributes and can be quite complex.

Figure 2 Graphical depiction of the Fåhraeus-Lindqvist effect demonstrating the decreasing relative viscosity of blood in relation to vessel diameter.
1.4.1 Regulation of macrocirculation blood flow

Control of blood flow within the macrocirculation has multiple components. However, the overall regulation of systemic arterial blood pressure and blood flow is independent of both local blood flow control and cardiac output. Within tissues or vessels that lack local autoregulation, changes in arterial pressure may result in dramatic effects on blood flow. If severe enough, perfusing pressure may fall below a critical closing pressure and lead to a complete cessation of blood flow. This may be of particular concern for venular flow. However, in normal resting conditions, there is a steady state sympathetic input creating partial contraction which leads to resting vascular tone, called vasomotor tone. Control of vasomotion is regulated by tissue oxygen concentration sensed by the vasomotor center in the brain. Within this vasomotor center are two secondary areas within the medulla, the vasoconstrictor area stimulating the sympathetic nervous system and the vasodilator area which functions by inhibition of the vasoconstrictor activity. There is also a sensory area which receives input from systemic circulation through the vagus and glossopharyngeal nerves leading to release of either parasympathetic or sympathetic tone to contribute to vascular tone. There are both acute and chronic controls of blood pressure; and these will be considered in relation to the body’s response to hypotension specifically.

With acute hypotension, there is a decreased stretch of the baroreceptors of the aortic arch and carotid bodies. This initiates the baroreceptor reflex, resulting in stimulation of the central nervous system vasoconstrictor center and inhibition of vagal parasympathetic tone. This causes an increase in sympathetic tone and activation of $\alpha_1$
adrenergic receptors which will then promote vasoconstriction and transfer of blood flow from one vascular segment to another. Increased sympathetic stimulation will also lead to increased chronotropy and inotropy, primarily via stimulation of the β₁ adrenergic receptors. While this will result in restoration of systemic arterial blood pressure, it will concurrently result in activation of vascular α₁ and α₂ adrenergic receptors and decreased blood flow to non-critical organs, including kidney, splanchnic organs, and resting skeletal muscles. Finally, an increased release of catecholamines (norepinephrine and epinephrine) will lead to enhanced sodium reabsorption in both the proximal and loop of Henle tubular segments which will secondarily cause water retention improving the vascular volume.

An additional mechanism in place to help maintain blood pressure is the chemoreceptor reflex. There are chemosensitive cells within the carotid bodies and aortic bodies. These cells respond to a variety of signals including decreased oxygen, increased carbon dioxide and/or increased hydrogen ions. With hypotension, activation of these cells leads to stimulation of the vasomotor center of the brain stem which leads to increased arterial blood pressure. Also under sympathetic influence, an increase in venous tone will result in an increase in venous return (decrease in venous capacitance) leading to an increase in cardiac preload. With the associated additional stretch of cardiac myocytes and increased force of contraction there is an increase stroke volume and cardiac output that will serve to raise mean arterial pressure. More chronic regulation of the macrocirculation generally occurs over a period of hours to days. A large component of chronic regulation involves the kidneys, which play a major role via
hormonal influence and blood volume regulation.\textsuperscript{1} This is primarily done through the renin-angiotensin-aldosterone system (RAAS) and increased antidiuretic hormone secretion, causing an overall retention of sodium within the kidneys and secondarily water which restores or maintains effective circulating volume.\textsuperscript{10} Angiotensin II and vasopressin also have vasoactive properties contributing to systemic vascular resistance and mean arterial pressure. However, additional consideration of the chronic maintenance of blood pressure is beyond the scope of this discussion.

1.4.2 Microcirculation blood flow regulation

While regulation of both macrocirculatory and microcirculatory blood flow has some dependency on systemic blood pressure, there is a significant component of local modulation that occurs at the level of the microcirculation. A key feature of this process is autoregulation, which functions to maintain local blood flow somewhat independent of the systemic pressure. Change in microcirculatory blood flow does not usually last for more than four hours, as tissue’s local autoregulatory mechanisms will override most vasoconstrictors.\textsuperscript{1} Autoregulation occurs through a variety of mechanisms, including responses to vascular tone, neuronal and hormonal signals, and through sensing of metabolic products.\textsuperscript{1, 6} The valuable function of the microcirculation, discussed later, requires that there is dynamic and constant regulation of the blood flow, based upon the individual needs of each tissue bed.\textsuperscript{6} If tissue demand for oxygen and nutrients is high enough, the ability of the cardiovascular system to increase cardiac output and provide adequate blood flow may be insufficient.\textsuperscript{1} The unique structure of the microcirculation allows for this local control both in both acute and chronic settings, maintaining the
microcirculatory blood flow during changes of the systemic arterial blood pressure between 70 to 175 mmHg.  

Arterioles are the most important effector for autoregulation, with the ability to change 50-60% of the total regulatory pressure within the vascular bed. Therefore, the arteriolar vascular resistance regulates microcirculatory perfusion. The smooth muscle within the wall allows for large changes of the vessel diameter, causing significant impact in the capillary beds with the ability to achieve up to 50% dilation or complete closure. Smooth muscle innervation with sympathetic fibers allows for rapid response to stimulation.

Capillary flow may also be dependent on or affected by vasomotion. Vasomotion is the rhythmic oscillations, determined by the precapillary sphincters, that occurs within the intermediate to small arterioles. This can lead to flow being intermittent through the capillaries. Depending upon tissue bed requirements, some microcirculatory systems are capable of super-regulation, where some capillaries have a 100% increase of flow, even when only a 25% reduction of perfusion pressure is sensed. This may lead to a lack of flow through other capillaries and further demonstrates the heterogeneity of the microvascular network.

Regulation of capillary perfusion is the ultimate goal of blood flow alterations through arterioles. Independent of autoregulation, some tissues may attempt to maintain perfusion through recruitment of capillaries, but this can also change capillary resistance resulting in further alteration of blood flow. With decreased perfusion and no autoregulation, capillaries may easily collapse in the absence of sufficient perfusing
pressure. Even a small change in capillary diameter may lead to marked increases in vascular resistance,\(^6\) adding a negative impact on blood flow that is already compromised. With reduction of blood flow, there may be aggregation of red blood cells which can alter their deformability\(^6\) and impact their ability to transverse the microcirculation.

In autoregulation, an acute local control generally occurs within seconds to minutes and may involve the arterioles, metarterioles and the precapillary sphincters.\(^1\) Although a lot of research has been done to determine the mechanism of this autoregulation, the changes are likely due to a combination of several mechanisms. The first mechanism is through active regulation with three types of specific inputs. The first type is myogenic theory which involves a change to smooth muscles and is independent of neuronal, metabolic or hormonal influences.\(^1,6\) Alteration of smooth muscle tone is in response to endothelial cells sensing a change in wall shear stress, leading to either vasodilation or constriction.\(^1,6\) Several mechanisms influence smooth muscle tone, including adjustment of sympathetic tone or hormones, such as norepinephrine or nitric oxide.\(^1\) With regards to nitric oxide, there are specific mechanisms that have been found to increase the release of nitric oxide and subsequent vasodilation. As blood transverses through the vessels, increased shearing of the blood past endothelial cells stimulates an intracellular elevation of calcium. This causes an increased production of nitric oxide within the endothelial cell which will diffuse to the vascular smooth muscle cell.\(^1\) Ultimately this leads to an increase in cyclic guanosine monophosphate causing relaxation of the muscle and secondary vasodilation through inhibition of intracellular
calcium within the myocyte. This adaptation of smooth muscle tone occurs most strongly in the arterioles but also can impact the arteries and venules and has a large influence on maintaining the basal vascular tone. However, it is self-limited to fluctuations of 20 mmHg and with a focus on protecting from elevations in perfusion pressure rather than preventing a fall in capillary pressure.

The second type of active regulation is metabolic, where the ability to sense various indicators of cellular metabolism will lead to local control of blood flow. One such example is local oxygen tension sensed by arterioles. With an increase in local perfusion pressure, arterioles will sense an increase in delivery of oxygen and nutrients. This will lead to vasodilation and a dilution of local vasodilatory substances, with then a countering vasoconstriction and normalization of blood flow. This local effect can be strong enough to override myogenic input. Additionally, oxygen tension and local metabolic conditions can be sensed through communication of the arterioles and adjacent venules, via a countercurrent exchange mechanism. This is especially helpful when there is increased oxygen utilization and demand by the tissue bed. As the tissue consumes the oxygen, this causes a decrease of oxygen within the venules which is then sensed by the arterioles. This leads to alteration of arteriolar tone, most strongly at the distal arterioles, although capillary and postcapillary regions can be impacted, causing vasodilation and subsequent increase delivery of oxygen to the tissues. Table 1 demonstrates some of the local mediators and the corresponding effects on the microvascular blood flow.
<table>
<thead>
<tr>
<th>Increased perfusion</th>
<th>Decreased perfusion</th>
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<tr>
<td>Carbon dioxide</td>
<td>Oxygen tension</td>
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<tr>
<td>Histamine</td>
<td>Endothelin</td>
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<tr>
<td>Potassium</td>
<td>Norepinephrine</td>
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<td>Hydrogen ions</td>
<td>Epinephrine</td>
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<tr>
<td>Glucose</td>
<td>Reactive oxygen species</td>
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<td>Adenosine</td>
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<td>Nitric oxide</td>
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<td>Inorganic phosphate</td>
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<td>Lactate</td>
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<td>Prostacyclin</td>
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<td>Prostaglandins</td>
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Table 1 Compilation of local mediators and respective impact upon the microcirculation blood flow when increased levels of the mediator are present.

The final type of active regulation includes hormones and nervous influence with two theories established to explain this type of regulation. One theory is the vasodilatory theory, where there is sensing of vasodilatory substances (Table 1). The most important substance is thought to be adenosine but also carbon dioxide, histamine, potassium, hydrogen, and glucose. The second theory is the lack of oxygen which causes relaxation of the precapillary sphincter and leads to vasodilation. Additional hormonal influence can be described as hormonal influence based upon delivery to the microvessels. Some are endothelial derived including nitric oxide causing dilation or endothelin which is a potent vasoconstrictor. Other hormones originate externally from
the microcirculation, including norepinephrine, epinephrine, angiotensin II, bradykinin, and histamine.\textsuperscript{1} Finally nervous input is primarily through sympathetic innervation. This innervation is strongest at the small arteries and arterioles but may have some impact at the metarterioles and precapillary sphincters.\textsuperscript{1} A small impact may occur at veins leading to increased blood volume but there is no innervation to the capillaries.\textsuperscript{1}

The second possible mechanism for autoregulation is a passive buffering of capillary pressure changes.\textsuperscript{6} This is possible in the microcirculation given its special structure and capillary network.\textsuperscript{6} The microcirculatory arcade and organization allows for a prevention of acute pressure changes within the capillaries.\textsuperscript{6} This may include the ability to recruit or collapse capillaries, or to dilate or constrict portions, resulting in maintenance of a relatively consistent pressure. The third mechanism described is passive regulation, which occurs from size and rheological changes within the venular portion of the microcirculation.\textsuperscript{6} Endothelial cells sense rheological changes, such as vascular factors, hematocrit, and viscosity, and signal the encircling smooth muscle to contract or to relax.\textsuperscript{3} Normally venules have little diameter change to effect autoregulation but if there is a change in venous resistance, it may have significant impact on capillary pressure.\textsuperscript{6}

While acute regulation of the microcirculation is important, long term control is vital for the health of the microcirculatory system. Acute autoregulation will only contribute to approximately 75\% of the vascular change needed for tissue regulation.\textsuperscript{1} Long term control is a slow and controlled process that involves the ability of vessels to adapt structurally and dynamically.\textsuperscript{1,6} This is accomplished by increasing the size or
number of blood vessels providing perfusion to various tissues through processes such as angioadaptation and/or angiogenesis. Some of the stimuli for long term control include blood pressure, blood flow, wall shear stress, or the metabolic state of tissue. This might be especially important in diseases such as chronic hypertension, chronic edematous states, diabetes or thrombotic states, all of which may lead to microvascular dysfunction chronically. While important for any microcirculatory bed, this mechanism is also vital for creation of collateral circulation. The combination of acute and long term regulation of the microcirculation contributes to maintenance of the microcirculation such that its vital function can continue.

1.5 Function of the circulation

The primary function of the macrocirculation is for delivery of blood to the microcirculation. It can further be stated that the microcirculation then has to perform what is arguably one of the most important functions of the body: provision of cellular oxygen and nutrients. Arterioles primarily function to regulate delivery of systemic blood flow into the capillary beds. Diffusion of nutrients, oxygen, and hormones, as well as removal of waste products (lactate, carbon dioxide), occurs at the capillary level, with each vessel expected to serve a surrounding tissue diameter ranging from 20 μm to 200 μm, depending upon its activity. This function is made possible by several features of the capillary bed, including the large cross sectional area and the low blood flow velocity. Exchange of these materials is highly dependent upon the vascular surface area, structure of the vascular wall, the permeability of the wall to the solute, and the solute’s concentration difference across the vascular wall.
The transport of the substances occurs through several routes including transcellular, transcellular via vesicles, and paracellular.\textsuperscript{6} Transcellular transport includes diffusion of water with dissolved substances through the capillary membrane via thermal motion.\textsuperscript{1} Lipid soluble substances, such as oxygen and carbon dioxide, can directly diffuse through cellular membranes.\textsuperscript{1} Transcellular transport may involve specialized vesicles, caveolae, which are useful for endocytosis and transcytosis of macromolecules.\textsuperscript{1} Paracellular transport may involve percolation of fluid through the intracellular cleft or special pores for movement of fluids, solutes, and macromolecules.\textsuperscript{1,6} Another method is via passive diffusion, either through transcellular or paracellular route.\textsuperscript{6} The diffusion distance is dependent upon tissue demand; as tissue demand increases, the distance from a capillary bed must be decreased.\textsuperscript{6} In some tissue beds, oxygen is the biggest limitation to the diffusion pathway.\textsuperscript{6}

Venules play an important role in the immune system response to inflammation or infection. Within the venules, leukocyte adhesion is accomplished through complex signaling pathways between leukocytes and the endothelium. Markedly selective adhesion molecules make venules the primary site for immune defense. In addition, venules feature low flow velocity and low wall shear stress which serves to facilitate leukocyte to margination to the vessel wall.\textsuperscript{6} Additionally, the actions of the red blood cells, such as central aggregation, will increase the margination of leukocytes.\textsuperscript{6}

During an inflammatory or pathological state, these features may change in the microcirculation, altering the function. Ultimately, several mechanisms can result in increased permeability of capillaries and venules, primarily through paracellular shifting
of fluid and solutes. Inflammation leads to contraction of the actinomycin within endothelial cells and a decreased adhesion between the endothelial cells. Histamine and serotonin released will increase the gaps between endothelial cells in both capillaries and venules. Other agents have been found to increase microvascular permeability including intracellular calcium, protein kinases, cyclic adenosine monophosphate, cyclic guanosine monophosphate, and nitric oxide. These mediators may bind to multiple receptors and lead to activation of a number of signal transduction pathways. Given the number of pathways and receptors, activation of inflammation may lead to a sustained increase in permeability through this self-perpetuating cycle. This makes targeting of these mediators a challenge and future therapy may require blocking multiple receptors.

A final aspect of the microcirculatory unit which is important for maintaining function and tissue fluid balance is the lymphatic system. While a full description of the structure and function of the lymphatic system is beyond the scope of this discussion, it is important to acknowledge its role in helping to control protein concentration, volume and pressure of interstitial fluid. The interstitium is made of a network of proteoglycan filaments, collagen fibers, and fluid created from capillary diffusion and filtration. The lymphatics are a large network of vessels running in tandem with arterial and venous circulation. They serve to remove proteins and large particulate matter, which cannot be reabsorbed into venules, and return them to the larger veins. The lymphatics consist of endothelial cells anchored to the interstitial connective tissue. Fluid, proteins, and particles will enter lymphatics through pores between endothelial cells and will be pumped away from the interstitium towards the larger lymphatic ducts. The pumping
action primarily occurs via successive valves but may be stimulated through contraction of surrounding skeletal muscle, movement of the respective body parts, adjacent arterial pulsation, or compression of the surrounding tissues.\textsuperscript{1} Therefore, maintenance of this space allows for the continued delivery of nutrients from the capillaries to the cells.

1.6 Indirect Monitoring of the Microcirculation

Given the importance of the microcirculation, evaluation or monitoring of this system has become a large focus of research. Unfortunately, depending upon the microcirculatory bed of interest, there may be significant limitations in the ability to accurately assess changes in microvascular perfusion. To help determine the extent of these limitations, and thereby the utility of more direct measurement of microvascular perfusion, studies have attempted to assess how well macrocirculatory monitoring (being much more readily available) correlates to microcirculatory changes.

1.6.1 “Upstream” macrocirculatory assessment

One method to evaluate the microcirculation can be through assessing ‘upstream’ features, measurements or factors that occur in circulation before the microvessels, such as blood pressure, stroke volume, and heart rate. However, global hemodynamics may fail to show regional and microcirculatory blood flow derangements. Further, microcirculatory dysfunction may occur earlier than documented macrohemodynamic changes.\textsuperscript{12} This disparity is likely due to the various mechanisms of local regulation which have already been described. A large number of studies have looked for correlation between the macrocirculation and the microcirculation, with somewhat conflicting results. One study evaluating changes in microcirculation versus
macrocirculation associated with septic shock found that there were similar macrohemodynamic measurements, including mean arterial pressure, central venous pressure, mean pulmonary artery pressure, cardiac index and heart rate, between the survivors and nonsurvivors groups.\textsuperscript{13} However, the survivors had improvement of microcirculatory parameters when shock was resolved while nonsurvivors did not\textsuperscript{13}; this demonstrated that macrohemodynamics may not reflect when microcirculatory health has been restored. Another group found that there were severe microvascular blood flow alterations in sepsis without affecting the global hemodynamic state, including mean arterial pressure and cardiac index.\textsuperscript{14} Finally, in evaluation of the sublingual microcirculation in severe sepsis and septic shock, there was no correlation between the microcirculation and the heart rates, mean arterial pressure, cardiac index or central venous pressures.\textsuperscript{15}

Additional studies looking at heart rate and cardiac index, found that there was no correlation with the microcirculation.\textsuperscript{16,17} One study found that measured delivery of oxygen did not have any correlation with the evaluation of blood flow in the microcirculation.\textsuperscript{17} Other studies evaluating the microcirculation found no correlation with central venous pressure.\textsuperscript{16,18} Evaluation of systemic blood pressure has been slightly more variable in results. Some studies did not find any correlation between the mean arterial pressures to microcirculation.\textsuperscript{16-18} However, one study found that in an early septic normotensive patient, no abnormalities of the microcirculation were found\textsuperscript{19}, which has not been the case for the majority of cases evaluating the macrohemodynamic changes and microcirculatory alterations. They suggested that for microcirculation
dysfunction to occur, there may need to be a concurrent severe impairment of systemic blood pressure.\textsuperscript{19}

\subsection*{1.6.2 Downstream macrocirculatory assessment}

Given the difficulty finding correlation with ‘upstream’ macrocirculatory variables, studies also evaluated for correlation with ‘downstream’ measurements. These parameters, such as lactate, central venous oxygen saturation, and tissue oxygenation, reflect perfusion and tissue utilization through the microcirculatory unit. These downstream factors are meant to evaluate the function of the microcirculation based primarily on measurement of oxygen utilization or presence of anaerobic metabolism. In sepsis, it has been found that there was no relationship between the microvascular parameters compared to the patient’s temperature, mixed-venous oxygen saturation,\textsuperscript{13, 14} or lactate.\textsuperscript{14} In a study of abdominal sepsis, the sublingual and intestinal microcirculatory changes did not have any correlation with the lactate measurements.\textsuperscript{16} In evaluation of two groups of patients after abdominal surgery (those who developed complications and those that did not), there was no difference in lactate or central venous oxygen saturation between the groups but there was an increased risk of developing complications associated with those demonstrating microvascular derangements.\textsuperscript{17} This group’s results additionally demonstrated that this occurred both for those patients with complications and those without.\textsuperscript{17} Another study found that sublingual microcirculatory alteration and organ failure was not accompanied by changes in central venous oxygen saturation.\textsuperscript{18} Edul et al found that utilization of oxygen may vary regionally and may not be detected systemically. This was achieved by demonstrating that oxygen transport parameters
failed to reflect both regional and microcirculatory blood flow derangements. This supports the notion that in some disease states, loss of local regulation can develop which may not be reflected in the ‘upstream’ or ‘downstream’ methods of patient evaluation.

1.7 Direct monitoring of the microcirculation.

Given the apparent disconnect that can exist, commonly used macrocirculatory parameters may not be ideal to determine the microcirculatory response in disease states as well as after resuscitation efforts. As a result there has been increasing interest in direct evaluation of the microcirculation in a variety of patient populations. There are important features of the microcirculation that would be ideal to evaluate. One such is nutritive tissue perfusion, which evaluates the density of perfused vessels and the flow within the vessels. Two parameters are used to describe the ability of the microcirculation to transport oxygen to tissues including microcirculatory flow and functional capillary density (FCD). Historically, FCD has been determined by direct microscopic measurement of the total length of red blood cell perfused capillaries per the observed area. Newer methods of monitoring FCD utilize computers to quantify the length of blood vessels, which is especially important in the irregular microvascular patterns.

There are some earlier methods which have tried to assess specific tissue microcirculation, including gastric tonometry, sublingual capnometry, and laser Doppler flowmetry. The theory of gastric tonometry is that the partial pressures of carbon dioxide and oxygen within the gastric lumen will be equal to that of the gastric mucosa. With this method, a gastric tube is used to measure gastric luminal carbon dioxide levels. While
carbon dioxide levels have been shown to correlate to tissue perfusion, there are several limitations to this method.\textsuperscript{20} These limitation include the need for a gastric decompression tube and the fairly labor intensive nature and steep learning curve which goes along with this technique.\textsuperscript{20} This method utilizes the assumption that mucosal and the arterial bicarbonate levels are the same which may not be true in pathological conditions.\textsuperscript{20} To avoid address some of the limitations associated with gastric tonometry, sublingual capnometry was developed to be less invasive, fast, and easy to use.\textsuperscript{20} This technique utilizes fiber optics to measure the levels of sublingual mucosal carbon dioxide.\textsuperscript{20} When comparing the gastric tonometry to sublingual capnometry, there was good correlation between the two methods in patient populations of septic or cardiogenic shock.\textsuperscript{21} This supports the notion that the much less invasive and user-friendly sublingual approach would be preferred over gastric.

However, limitations of using these methods for determining tissue blood flow lead to development of laser Doppler flowmetry (LDF). The LDF method determines the velocity of the moving red blood cells via the Doppler frequency shift; essentially when the light is scattered, the machine determines its frequency caused by tissue blood flow and estimates the speed of red blood cell movement.\textsuperscript{3} While this method has led to improved microcirculatory evaluation, the drawbacks, including the inability to determine the depth of tissue being assessed, have necessitated development of more advanced technology for tissue blood flow monitoring.\textsuperscript{3} However, since the newer methods can only be used on mucosal surfaces, laser Doppler flowmetry is still used clinically to evaluate flow, especially on cutaneous surfaces.\textsuperscript{22-24}
Some of the newest technology enables bedside use and overcomes the previous methods’ shortcomings. The two main methods, orthogonal polarized spectral (OPS) and sidestream dark field microscopy (SDM), evaluate the microcirculation through dark field imaging. Both techniques utilize emission of polarized green light (530 nm wavelength) to create an image of the microcirculatory network, as depicted in Figure 3. Hemoglobin absorbs this wavelength of light while the other structures reflect the light back to the device. The information is then gathered by a receiver and sent to a computer monitor as video images, which can be evaluated during the image collection and stored for later examination. This allows for outlining of the microcirculation as well as determination of flow characteristics through these vessels. The OPS method does have limitations, mainly blurring and suboptimal capillary imaging caused by motion artifact. To overcome this, SDM was created to allow for enhanced image quality. When compared to OPS, a strong correlation between the two monitoring devices was found. Figure 4 represents the comparison of microcirculation imaging for SDM (referred to as SDF) and OPS units.

However, SDM had superior image quality, primarily though higher image contrast, and found to have improved clinical applicability. Enhanced image quality with SDM is through improved focusing depth without interference of deeper vasculature lowering image quality as with OPS. There is also a noticeable improvement of the capillary contrast and increased resolution of flowing red blood cells with the SDM unit, which might be attributed to the stroboscopic illumination of the light source. A final improvement of the SDM technology for clinical use is its ability to be powered with a
battery and portable computer, making it easier to use bed-side compared to OPS which has a strong light source requiring a large power source.\textsuperscript{25} For all its improvement in visualization of microcirculation, SDM does have limitations including utilization of only mucosal or serosal surfaces, interference from pigmentation,\textsuperscript{26} motion artifact, contact pressure altering blood flow or image quality, and impact of surface moisture levels,\textsuperscript{3,25}

Figure 3 Picture depiction of the functional unit of the SDM unit (referred to as SDF) for imaging of the microcirculatory bed. As demonstrated, a green light from the SDM unit is absorbed by the hemoglobin and reflected by the remainder of the tissue. This is then transmitted electronically to a connected monitor and allows visualization of the microcirculation in real-time.
To obtain the most representative information of the tissue bed of choice, and report microvascular data in a consistent and repeatable manner, a group of experts created a consensus approach regarding microcirculatory imaging. It was recommended to obtain at least five videos of adjacent sites in the region of interest with the goal of then having at least three quality videos of twenty second duration.\textsuperscript{3,27} The tissue should have excessive secretions removed and adequate focus and contrast should be adjusted.\textsuperscript{3,27} To avoid pressure induced artifact, venular flow within the image should be continuous.\textsuperscript{18,27} The overall requirements for videos of quality must have adequate duration, focus, content, image stability, and pressure.\textsuperscript{3,28} For representative evaluation of the microcirculatory images, a scoring method was created utilizing the density of vessels and the character/quality of blood.\textsuperscript{27} Total vessel density (TVD) is the total number of vessels found to cross arbitrary lines of a grid superimposed on the image.\textsuperscript{27} After flow characteristic is assigned to each vessel (no flow = 0, intermittent flow = 1,
sluggish flow = 2, normal flow = 3, hyperdynamic flow = 4), proportion of perfused vessels (PPV) is calculated from the total number of vessels minus the vessels with only intermittent flow or no flow at all, divided by the total number of vessels and then multiplied by 100.27 Perfused vessel density (PVD) is the number of vessels that are being perfused,27 and is calculated by multiplying TVD by PPV.29

Figure 5 below, demonstrates vessels outlined by red blood cells which are black. During video capture, the individual red blood cells would transverse through the microcirculation and the flow quality characterized.30 As a summary characteristic, PVD equates to the FCD and offers the best overall assessment of tissue perfusion.27 Finally, the microvascular flow index (MFI) determines the predominant flow type in the microcirculatory bed by dividing the visual field into quadrants (Figure 6).27 The predominant flow types are labeled as no flow = 0, intermittent flow = 1, sluggish flow = 2, and normal flow = 3. The consensus group found that PVD, PPV, and MFI were all parameters to allow for comprehensive description of microcirculatory vascular bed perfusion.27
Figure 5 Above is shown a still image of the microcirculation demonstrating the perfused vessels, outlined by the red blood cells shown as black.

Figure 6 Demonstrates the method of determining the microvascular flow index, with a SDM video divided into four quadrants. Each quadrant would then be assigned a predominant flow index; 0 = no flow, 1 = intermittent flow, 2 = sluggish flow, and 3 = normal flow.

Although this scoring system allows structured and uniform analysis, there is debate about intraobserver variability, given the somewhat subjective nature of the vascular analysis. One study evaluated this and found good intraobserver correlation for
TVD, PVD, and PPV; MFI was less reliable.\textsuperscript{31} This study found that even with repeated analysis, there was good repeatability in vessel detection and flow classification.\textsuperscript{31} The biggest downfall to SDM is the length of time needed for video analysis. Although subjective evaluation of the microcirculation can be done at the time of video collection, no official scoring system is in place for rapid analysis. One study did evaluate if a rapid bedside scoring system of video quality could be done to decrease the number of poor quality videos and found the largest failure was pressure artifact impacting the quality.\textsuperscript{28} Another group compared bedside evaluation to the information gained through typical data analysis.\textsuperscript{32} They found no correlation between their subjective bedside scores and the official analysis; their results make the bedside an unsuitable method for qualitative microcirculation assessment.\textsuperscript{32} Therefore, it is still recommended that quantitative analysis is performed off-line with the available software program (Automated Vascular Analysis). While it is reported that analysis can be performed in as little as 10 minutes, published studies have suggested times of more than 30 minutes (to an hour) for each video.\textsuperscript{26,29} Regardless of the time delay in video analysis and other potential limitations, SDM has been used in numerous studies for microcirculation evaluation.

\textbf{1.8 Examination of microcirculation in disease}

The microcirculation has a variety of changes that can occur in response to diseases. One major area of change is the endothelium. The endothelium is a target for cytokines, including interleukin-1 and tissue necrosis factor $\alpha$, which contribute to alteration of the increased adhesion leukocytes.\textsuperscript{2} Shock can lead to endothelial cell injury and swelling which may cause mechanical obstruction of microvessels and further
worsening perfusion and flow. The endothelium may also have decreased response to catecholamines, increased response to vasodilators and increased release of dilator substances, especially hydrogen and potassium ions. Additional changes to the microcirculation in disease may be secondary to white blood cell activation. Some of these effects may include increased vascular resistance, capillary plugging from platelets and leukocytes, and release of cytokines and oxygen radicals causing an increased permeability. While knowledge of these alterations have been found experimentally, evidence in clinical patients would be helpful in diagnosis, prognosis, therapeutic response, and recovery.

1.8.1 Microcirculation in the gastrointestinal tract

Evaluation of the microvascular perfusion has been performed in a number of diseases, including cardiogenic shock, post-cardiac arrest, high risk surgery and sepsis. Given its potential role as a sentinel, and potential contributor to disease progression, the gastrointestinal tract has been a focus for microcirculatory evaluation. The gastrointestinal tract is in a constant state of controlled inflammation, including inflammatory cell traffic in the mucosa. When inflammatory conditions occur in these tissues, they are characterized by increased recruitment of inflammatory cells and a dysregulated activation of infiltrating cells leading to tissue injury and organ dysfunction. When evaluating the microcirculation in these inflammatory conditions, alterations documented include dilation and tortuosity of vessels, loss of normal vascular tapering, an increase in vascularity, and congestion of mucosal and submucosal microvessels. Studies have used the gastrointestinal tract to assess response of the
splanchnic circulation to a shock state. It has been found that there is a progressive vasoconstriction at all levels of the intestinal microcirculation causing intestinal mucosal injury and dysfunction of the mucosal barrier.\textsuperscript{2} For further evaluation of the intestinal microcirculation in diseased intestine, laser Doppler flux and tissue oxygen tension were evaluated in a pig model of necrotic colonic tissue.\textsuperscript{36} It was found that assessment of the microcirculation could predict necrosis when at least 30\% of the mucosal thickness was affected.\textsuperscript{36}

Several studies have utilized SDM technology for evaluation of gastrointestinal microcirculation. One study evaluated normal rat skeletal muscle and ileal mucosal microcirculation.\textsuperscript{30} A second experimental study demonstrated a significant decrease of intestinal microcirculatory blood flow during cardiac arrest. This technology has also been used to evaluate small intestinal microcirculation and response to therapy, with prevention of microcirculatory derangements in experimentally induced sepsis,\textsuperscript{37} reduced sepsis initiated microcirculatory dysfunction\textsuperscript{38} and surgical stress and pain.\textsuperscript{39} Another study in a sheep model of induced septic shock found that ileal microcirculation decreased and was not improved with the addition of norepinephrine.\textsuperscript{40}

\textbf{1.8.2 Comparison of sublingual microcirculation to gastrointestinal}

While the gastrointestinal tract is a very useful tissue bed to evaluate complex responses of microvessels in disease states, the difficulty in accessing intra-abdominal organs limits utility in most diseases (short of intra-operative assessment). However, the sublingual microcirculation has several features that make it a possible surrogate for difficult to monitor regions, especially the gastrointestinal tract. The region is easily
accessible and has common embryogenic origin with the intestines.  Several studies have exploited the sublingual microcirculation for the gastrointestinal tract and have found good correlation for a variety of microcirculatory evaluation methods.\textsuperscript{16, 18, 33, 34, 41, 42} One illuminating study evaluated the relationship between sublingual and intestinal microcirculation with OPS one day and three days after diagnosis with abdominal sepsis.\textsuperscript{16} There was no correlation between the two sites’ microvascular indexes or hemodynamics on the first day of sepsis.\textsuperscript{16} However, by day three there was return of correlation between the sites and restored flow at both regions.\textsuperscript{16} Another study evaluated circulatory shock, caused by a combination of sepsis and hypovolemia, with OPS and tissue partial pressure of carbon dioxide and found that there was progressive decreased capillary blood flow in both tissue beds that correlated with the severity of tissue ischemia.\textsuperscript{34} Finally, a study evaluating the early changes in microcirculatory blood flow in sepsis found an association with organ failure in first 24 hours when microcirculatory dysfunction was present.\textsuperscript{18} Further, the study demonstrated improvement of microcirculatory flow was associated with a decrease in organ failure.\textsuperscript{18} Regardless of the study performed, it has been acknowledged that the sublingual microcirculation can be used for detecting and evaluating microcirculation of the gastric mucosa independent of the systemic hemodynamics and oxygen derived variables.\textsuperscript{33}

\textit{1.8.3 Use of SDM in Veterinary Medicine}

The majority of the literature on microcirculation evaluation has been done in human diseases. However, more recently a number of studies have been performed in veterinary medicine to explore the potential application of this technology. Silverstein et
al used SDM to establish normal microcirculatory variables in canine mucogingival tissue. Studies following this paper also included evaluation of hemorrhagic shock in a canine model. One found significantly decreased microcirculatory variables with induction of hemorrhagic shock and a lack of correlated hemodynamics except for mild correlation between PVD and heart rate. A second study of hemorrhagic shock in canines evaluating intestinal microcirculation found a decrease in all microcirculatory variables with hemorrhage but improvement in these variables with treatment of a hyperviscous solution. Another study, recently published, looked at the impact of various crystalloid infusion rates on microcirculation while under anesthesia. It was found that fluid rate had no impact on perfusion for those vessels of less than 20μm in diameter. The first study of feline microcirculation is also the only known veterinary publication using the sublingual microcirculation, demonstrating that it is a feasible tissue bed for examination in veterinary medicine. Yet another study evaluated equine colonic microcirculation after torsion and de-rotation, demonstrating good correlation between microcirculatory parameters and changes on histopathology.

When considering the use of microcirculation imaging in veterinary patients, several limitations must be discussed. One limitation, discussed previously, is the time needed for video analysis. A bedside evaluation model was created to minimize the time needed for video analysis. The group looked at video quality including stability, content, illumination, focus, and pressure and three perfusion parameters of classic SDM evaluation (TVD, PPV, MFI) and a new SDM evaluation of capillary vessel density (CVD). When comparing rapid subjective assessment of video quality to more detailed
image examination, there was reasonable agreement. Additionally, the ability to repeatedly draw the same conclusions regarding image quality did not change with time nor did agreement between the bedside evaluation and computerized vascular analysis. This group concluded that rapid bedside evaluation of image quality was possible, but there was a lack of correlation between “bedside” quantitative assessment of microvascular parameters and determination with vascular analysis software. Therefore, future examination of a bedside method of quantify microvascular perfusion is needed.

Another limitation for veterinary medicine is gaining access to the tissue beds of interest. The buccal mucosa represents the most readily accessible tissue bed in dogs and so has been explored the most thus far. While this tissue is easy to access, patient tolerance for probe placement be limited, depending on patient compliance and degree of compromise. Additionally buccal mucosa may not represent other tissue beds within the body. To improve patient compliance, many of the studies evaluating microcirculation have been performed under general anesthesia, which may alter microvascular perfusion and thereby accurate assessment of microcirculatory parameters. As in many veterinary prospective studies, small sample sizes are an inherent limitation. A final limitation for veterinary medicine is the availability of the SDM unit for clinical or experimental use, as it is costly equipment, requires computer access, and requires specialized software. While these papers have evaluated microcirculation for a small number of disease processes, a large number of diseases remain to be studied and are a future focus for veterinary medicine.
1.9 Gastric dilatation volvulus

Given the amount of research demonstrating the feasibility of utilizing SDM technology on intestinal disease, using this method for evaluation of common canine disease is a tempting research opportunity. One such disease is gastric dilatation volvulus (GDV) which is common disease in dogs with a reported occurrence of 2.4 to 7.6 dogs per 1000 admissions.\textsuperscript{47} Certain breeds have been found to be at higher risk of GDV including Great Danes and German Shepherds.\textsuperscript{47} Although several risk factors have been identified in a variety of dog breeds, the etiology remains unknown. While the disease process itself is fatal without emergency surgical intervention, there are several secondary consequences that may occur including tissue hypoxia, gastric necrosis, systemic inflammatory response syndrome, sepsis, peritonitis, disseminated intravascular coagulopathy and death.\textsuperscript{48-50} Several studies have shown variable mortality, with variable rates reported as 10% to 33%.\textsuperscript{47, 51-54} Potential risk factors for non-survival include the duration of pre-admission clinical signs, development of acute renal failure, need for splenectomy, presence of gastric wall necrosis, and untreated gastric wall necrosis.\textsuperscript{48, 55} Gastric necrosis has been reported in 10% to 25% of cases\textsuperscript{52-54, 56, 57} and has been associated with increased risk of secondary complications including multiple haemostatic abnormalities\textsuperscript{58} and increased mortality rate.\textsuperscript{51-53, 55, 58, 59} A recent study found macroscopic gastric wall necrosis in 19% of the GDV patients and off these patients, 62.5% did not survive to discharge.\textsuperscript{60} Further, all dogs that died had gastric necrosis.\textsuperscript{60} Despite advancements in medicine and surgical management, the mortality rate, especially when associated with gastric necrosis, remains elevated.
Intra-operative evaluation of gastric necrosis is a subjective assessment based on stomach wall thickness and texture, serosal surface color, presence of peristalsis, and serosal capillary perfusion.\textsuperscript{51, 56, 60, 61} However, this subjective assessment of gastric wall necrosis may not accurately reflect tissue viability, especially as it relates to prognosis.\textsuperscript{60} Further, it may be misinterpreted in 40\% of cases leading to incomplete removal of compromised or nonviable gastric wall with increased risk of stomach rupture or surgical dehiscence.\textsuperscript{56} Historically, there has also been concern that mucosal necrosis, which cannot be evaluated intra-operatively, is often more extensive than serosal necrosis.\textsuperscript{62} With this concern, methods to more directly assess intra-operative gastric microcirculation have been explored in both human and veterinary medicine.

Two studies have been performed evaluating the effects of increased intra-abdominal pressure on the tissue microcirculation. One study used an animal model and intravital video microscopy with a stepwise increase in intra-abdominal pressure; at 10mmHg and 15mmHg, there were concurrent stepwise decreases in mucosal perfusion, functional capillary density and red blood cell velocity.\textsuperscript{63} Therefore, with increasing intra-abdominal pressure, there was impairment of the mucosal microcirculation, postulated to be due to increased vascular resistance through mechanical compression of the vascular bed causing intestinal hypoperfusion.\textsuperscript{63} Another study using laser Doppler flowmetry evaluated the gastric mucosa microcirculation with experimentally induced increased intra-abdominal pressure.\textsuperscript{64} It was found that with increasing pressure, there was a similar decrease in gastric, renal and small intestinal microcirculation, with the gastric serosa most effected.\textsuperscript{64} These studies have demonstrated the susceptibility of abdominal
organ microcirculation to intra-abdominal pressure, making it also possible that increased pressure within that organ may also cause microcirculatory compromise, such as in gastric dilatation volvulus.

Perhaps most closely approximating the likely changes in that might occur with GDV, an examination of large colon volvulus in equine patients has been performed.  Using SDM technology in both experimental and naturally occurring disease, the colonic microcirculation was significantly altered.  When compared to histopathological samples, the microvascular perfusion indices were predictive of tissue of necrosis.  An older paper evaluated gastric blood flow in both experimentally induced and naturally occurring GDV in dogs.  Using laser Doppler flowmetry and injected microspheres, gastric capillary blood flow was measured.  It was found that those patients had significantly reduced capillary blood flow, by approximately 69%, when portal hypertension and gastric ischemia were present.  For the GDV patients that required gastric resection, there was a significantly lower capillary blood flow than in the dogs that did not have resection.  When evaluating the blood flow with injected microspheres, there was not only low blood flow but also a low concentration and/or aggregation of the microspheres, which may demonstrate additional complications that arise from the disease process that contribute to poor microvascular perfusion.  Despite these findings by Monnet et al, some diagnostic limitations were revealed, including evaluation of only a small volume of tissue directly beneath the probe and interference of beam depth penetration by tissue edema.  Further, this diagnostic tool does not provide a visual assessment of the microcirculation.
1.10 Study rationale

While studies have demonstrated continued advancement in technology, there is a paucity of information on canine gastric microcirculation, both in normal patients and in those with disease. Our studies collectively have several objectives: to demonstrate the feasibility of microvascular assessment of canine gastric serosa using SDM technology; to establish normal microcirculatory indices for canine gastric serosa; to compare and correlate gastric microcirculation to sublingual microcirculation; to compare and correlate systemic variables to microvascular parameters; and to evaluate the sublingual and gastric serosa microcirculatory alterations in naturally occurring gastric dilatation volvulus. To the authors’ knowledge, studies of this nature have not been performed and have the potential for great clinical significance. With this advancement, it is our hope that in future cases, surgeons may use microcirculation data to improve determination of gastric tissue viability intra-operatively.

1.11 References
18. Trzeciak S, McCoy JV, Phillip Dellinger R, et al. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with


Chapter 2: Microcirculatory assessment of canine gastric serosa during elective gastropexy using sidestream dark field microscopy

2.1 Introduction

The microcirculatory unit (composed of arterioles, capillaries, and postcapillary venules measuring < 200 um) is responsible for controlling the distribution of blood flow within organs and maintaining tissue perfusion.\(^1,2\) During disease states, local regulation may be lost with resultant impairment in tissue perfusion and oxygenation. Given that there are differences in local versus systemic circulatory control, changes in the microcirculation may not be reflected by measurement of standard systemic variable parameters.\(^1\) These parameters may include mean blood pressure, heart rate or cardiac index, and have been demonstrated to have poor correlation to microcirculatory changes in a number of studies.\(^3\text{-}7\) As such, direct evaluation of the microcirculation has become increasingly appealing with significant potential for clinical relevance in both human and veterinary medicine. In people, monitoring of microvascular perfusion has been performed in a number of clinical diseases including sepsis, shock, surgical complications, trauma, and cardiac.\(^2\text{-}9\) In veterinary medicine, there has been limited investigation in both health and disease states.\(^10\text{-}15\)

While there are several available methods to image the microcirculation, sidestream dark field microscopy (SDM) has gained popularity because of superior image quality and improved clinical utility.\(^16\) This method is more thoroughly described
elsewhere\textsuperscript{13, 14, 16, 17} but briefly, the techniques utilize emission of a green light (wavelength 530 nm) that is absorbed by hemoglobin and reflected by all other tissues. The device then captures the reflected light and creates real-time video images of flowing erythrocytes, thereby outlining the microvessels and allowing assessment of vessel density and perfusion. Previous studies have evaluated blood flow and/or microcirculation within the canine gastrointestinal tract.\textsuperscript{11, 12, 18} One such paper utilized laser Doppler flowmetry to evaluate blood flow within the gastric serosal surface in normal dogs with portal hypertension and gastric ischemia as well as in dogs with clinical gastric dilatation volvulus.\textsuperscript{18} However, given the more recent advancement of technology and improved ability to assess the microcirculation, SDM has replaced laser Doppler flowmetry both experimentally and clinically. Recent veterinary studies utilized SDM technology on intestinal serosa\textsuperscript{12, 13} and buccal mucosa\textsuperscript{10, 14, 15} demonstrating the feasibility of the SDM on mucosal and serosal surfaces. Human studies have demonstrated good correlation between oral cavity (sublingual) and gastrointestinal microcirculation,\textsuperscript{1, 6, 19-21} suggesting this more readily accessible site might serve as an acceptable surrogate in veterinary patients as well. However, no veterinary studies have investigated direct assessment of gastric serosal microcirculation with SDM, or the potential relationship with that of the oral mucosa, especially the sublingual tissue beds.

The goal of this study was to establish a technique for intraoperative assessment of sublingual and gastric serosal microcirculation using SDM. We further sought to determine gastric serosal microcirculatory parameters for healthy anesthetized dogs and evaluate correlation with sublingual mucosa. A secondary goal was to evaluate
correlation between systemic variable parameters and microcirculatory variables at both locations. Our hypothesis was that there would be good correlation between gastric serosa and sublingual mucosal microcirculations but a poor correlation when either is compared to systemic variable parameters.

2.2 Materials and Methods

2.2.1 Animals

The study protocol was approved by The Ohio State University Veterinary Medical Center Institutional Animal Care and Use Committee. Fifteen apparently healthy client-owned canines presenting for prophylactic gastropexy from May 2013 to February 2014 were enrolled. Informed consent was obtained from owners prior to enrollment. Dogs were considered to be healthy based upon history, physical examination and laboratory findings performed at the discretion of the attending clinician. At minimum a packed cell volume and total solids was performed prior to anesthesia. Exclusion criteria included history of gastric dilatation within 48 hours, history of any pre-existing disease conditions, owner decision for laproscopic gastropexy, or failure to obtain owner consent.

2.2.2 General anesthesia and instrumentation

Premedication protocol included acepromazine\textsuperscript{a} and a mu-opioid agonist\textsuperscript{b,c,d} with dosing calculated at the discretion of the anesthesia service. A peripheral intravenous catheter was placed. The induction protocols included propofol\textsuperscript{e} (n=10) or propofol and ketamine\textsuperscript{f} (n=5) with dosing to effect to allow intubation. All patients were placed under general anesthesia which was maintained with isoflurane\textsuperscript{g} inhalant and 100% oxygen.
After routine surgical preparation for an abdominal exploratory surgery, all patients were transported to the surgical suite and placed in dorsal recumbency. All patients were connected to a hemodynamic monitor, as described below, and standard surgical thermal support provided at the discretion of the anesthesia staff. All patients received intravenous crystalloid fluid therapy at the discretion of the anesthetist. A routine laparotomy was performed with abdominal exploration. If performed, ovariohysterectomy or orchiectomy was completed either before or after gastropexy based on surgeon preference.

2.2.3 Measurement of systemic variable parameters

The systemic variable data consisting of heart rate via electrocardiogram, respiratory rate, noninvasive blood pressure, end-tidal carbon dioxide (EtCO₂), temperature and pulse oximetry were recorded at the time of video capture for both the sublingual mucosa and the gastric serosa. Noninvasive blood pressure measurements were performed immediately after anesthesia induction via Doppler and the intraoperative measurements were performed via oscillometric methodology. Temperatures were obtained via esophageal temperature probe. A single lactate measurement (sample obtained from the jugular vein) was performed immediately after obtaining images of the gastric serosa.

2.2.4 Microcirculatory video capture

All SDM video images were obtained using the Microscan sidestream dark field microscopy unit with a specifically designed probe cover (Figure 7). The Microscan unit was connected to a laptop and monitor, allowing for real-time video display. At the time
of acquisition, videos were assessed for clarity, stability and evidence of compression artifact. Adjustments were performed to ensure optimum image quality, as previously described. If there was question regarding quality, if there was significant movement, or fluid/blood obscured the field, additional videos were obtained. Based upon previous established consensus criteria, at least three videos of 20 second duration is recommended for optimal analysis at each tissue bed. Videos were acquired until it was believed that this was achieved at each tissue bed. The number of videos acquired and total time to obtain videos were recorded.

**Sublingual mucosa**

In previous reports of SDM use in dogs, the buccal mucosa was used to obtain the microcirculation images. However, in pilot investigations, it was determined that patient positioning during anesthesia (dorsal recumbency) and tissue pigmentation made it difficult to consistently obtain quality buccal images. As such, it was decided to examine the sublingual mucosa, given that this area has been utilized in human medicine, and successfully explored in feline patients. In order to minimize impact on anesthesia time, sublingual videos were obtained either before (if ovariohysterectomy/orchiectomy performed first) or after (during gastropexy) the gastric videos based on optimal timing for access to the stomach (see below). To acquire the sublingual images, the microscan probe was lightly placed against the base of the dog’s frenulum and braced against the table to minimize motion (Figure 8). Optimum tissue moisture and probe contact was ensured (based upon image quality) via gentle removal of excessive saliva or application of saline.
**Gastric serosa**

To maintain intra-operative sterility while obtaining videos, one investigator performed routine procedures for surgical scrub, gowing and gloving. A long sterile palpation sleeve was used to cover the SDM hand-piece and the proximal portion of the data cable at risk of contact with the surgical field. In addition, the probe itself was covered with a new sterile cover (Figure 9). During preliminary attempts, it was discovered that significant condensation accumulated between the probe and the cover resulting in diminished image quality. It was suspected to be due to the change of the probe surface from room temperature to body temperature in the abdominal cavity. To limit this effect, the probe was pre-warmed in the axilla of a co-investigator leading up to intraoperative use and an anti-fogging solution applied to the probe end prior to attachment of the sterile cover.

The gastric serosa videos were obtained from a consistent location on the fundus toward the greater curvatures of the stomach (Figure 10). Videos were obtained with the stomach within the abdomen and stabilized using minimal tension on stay-sutures preplaced for the gastropexy and remote from the site of probe placement. Any blood pooling in the area was lightly blotted away with a moistened gauze. During video acquisition it was not possible to obtain 20 second videos without some motion from peristalsis and/or ventilation, and so efforts were continued until at least 3 minimally affected videos were obtained. If after 10 minutes it was not possible to acquire suitable videos, efforts were discontinued to limit impact on anesthesia time.
Figure 7 Picture of the Microscan® SDM hand unit with the specifically designed probe cover.

Figure 8 Picture of sublingual SDM video obtainment of anesthetized patient in dorsal recumbency.
Figure 9 Intraoperative picture of the SDM unit with sterile preparation obtaining gastric serosa images.

Figure 10 Figural depiction of portion of gastric serosa for obtaining microcirculation images.
2.2.5 Microcirculatory video analysis

After all study videos had been acquired, the videos were assessed for quality (image resolution, motion, and the presence of pressure artifact) by one investigator (ESC). During this assessment it was determined that motion created by peristalsis and ventilation, or pooling of blood, precluded the ability to assess steady gastric serosa videos for the recommended 20 seconds. The longest consistent period without motion or interference was 5 seconds. As such, all videos (gastric and sublingual) were trimmed to that time frame. If 3 quality videos of at least 5 seconds duration were still not available, the subject was withdrawn from analysis. The three videos of best quality from each subject/location were renamed, randomized and moved forward to vascular analysis.

Vascular analysis was performed to determine microcirculatory variables (as previously described based on consensus criteria\textsuperscript{11, 22}) using software\textsuperscript{9} specifically designed for SDM.\textsuperscript{11, 22} Briefly, this includes total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD), and microvascular flow index (MFI). Detailed descriptions of video analysis and how these values are determined can be found in numerous sources.\textsuperscript{11, 13, 22} Two separate investigators (KD and AD) each analyzed the screened videos. The investigators were unaware of the patient identity, video capture location, or the measurements obtained by the other investigator. To assess repeatability of the vascular analysis, intraobserver and interobserver variability was determined for all microvascular variables. For intraobserver variability, eight subjects were randomly selected; one video each for sublingual and gastric microcirculation was then analyzed in triplicate by two investigators (KD and AD). Microvascular parameters
from the investigator with the lower intraobserver variability would be used for the rest of the data analysis.

2.2.6 Statistical analysis

The independent variables included the measured systemic variables, as well as the determined microvascular parameters (TVD, PPV and MFI) and one dependent calculated variable (PVD). These variables were obtained from the measurements of a single, trained observer (KD). Dependent variables were evaluated for normality both graphically and with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Due to non-Gaussian distribution of some variables, all data are reported as medians (range). Dogs received one of two anesthetic protocols – propofol only or propofol and ketamine. The dependent variables were evaluated for differences between the two anesthetic protocols using a Mann Whitney rank sum test. Because no statistically significant differences were identified between the two anesthetic groups for any of the variables, the dogs were combined for further analysis without regard to anesthetic assignment. The four dependant variables were subsequently compared across the two sites of measurement (gastric versus sublingual) using a Mann Whitney rank sum test. Potential correlations between the dependent variables and systemic variables were explored using a Spearman’s correlation. Due to the exploratory nature of these correlations, Bonferroni corrections were not applied.

Intraobserver variability of microvascular data for eight randomly selected videos (four gastric and four sublingual) was evaluated for repeatability with a coefficient of variation as previously described. Interobserver variability was determined for the
averaged microcirculatory variables in the thirteen dogs using Bland-Altman analysis, as previously described.\textsuperscript{24} The interobserver variability results are reported as bias (limits of agreement). The percent error was calculated as 1.96 SD/mean value, with <30\% representing clinical repeatability.\textsuperscript{25}

Statistical analyses were performed with commercially available computer software\textsuperscript{f}. For all analyses, p < 0.05 was considered significant. Based on previous studies\textsuperscript{11, 12} a sample size of 12 dogs was predetermined to provide a statistical power of 80\% to detect a difference of 5\% for PPV between two sites (assuming SD of 4.2\%; alpha=0.05).

2.3 Results

A total of 15 dogs were enrolled but upon video analysis two dogs were excluded due to inability to obtain 3 gastric videos of sufficient quality for analysis. Therefore 13 dogs were included for data analysis. The dogs had a median age of 0.75 years (0.5 to 6 years). The mean weight was 33.3 kg (±10.8). Nine dogs were female (8 sexually intact; 1 spayed) and 4 were males (3 sexually intact; 1 castrated). The subject breeds consisted of 6 Weimaraners, two each of German Shepherd, Newfoundland, and Great Dane, and one Shiloh Shepherd. The mean packed cell volume was 47.0\% (± 4.5) and mean total solids was 7.0 g/dL (± 0.7). A total of five patients were within the ketamine and propofol group and eight patients were within the propofol only group.

There was a significant learning curve for acquiring suitable videos at both locations, with more challenges at the gastric site. A total of 83 sublingual videos (average of 5.5 per patient), as depicted in Figure 11, were acquired with at least three
quality videos for all patients at that location. At the gastric location, Figure 12, a total of 79 videos were acquired (average of 5.3 per patient). Of these, two patients did not have any videos of sufficient quality. Therefore, a total of 84 quality videos out of the desired 90 (15 subjects, two locations, 3 videos each) were obtained (93%). The most common cause of poor quality was lack of video stability, likely due to gastric peristalsis and movement secondary to patient ventilation. Total average time to acquire videos for each patient was 4.9 (±1.4) minutes for sublingual and 4.9 (±2.8) minutes for gastric. To assess impact of experience on acquisition time, this was further divided into early subjects (first 7) and late subjects (remaining 8). Comparing time to obtain sublingual videos between the early and late subjects, there was no statistical difference, with a mean time of 5.28 (±1.70) minutes and 4.62 (±1.30) minutes respectively. However, video acquisition time for gastric videos was significantly different with a mean of 6.7 (±3.4) minutes for early patients compared to 3.38 (±1.19) minutes for the later patients.

The descriptive data of both systemic variables and microcirculatory variables are summarized (Table 2). There was no statistically significant difference between the ketamine and propofol and propofol only groups for any systemic or microcirculatory variables at either location (data not presented) and so the remainder of the statistical analysis was performed with the combined data. There was no significant difference between systemic variables when comparing those obtained at the same time as the sublingual videos to those obtained during gastric video acquisition. When comparing microcirculatory parameters between the sublingual and gastric tissues, both TVD and
PVD were found to be significantly different (Figure 13). The remainder of the microcirculatory variables was not statistically significant.

Determination of correlation between gastric and sublingual microcirculation, as well as systemic variables (Table 3) and the microvascular data are reported. There were negative correlations between sublingual TVD and EtCO₂, PVD and respiratory rate, PPV to respiratory rate and systolic blood pressure, and MFI and temperature. Correlations with gastric microcirculation were also negative between TVD/PVD and pulse oximetry and EtCO₂, as well as PPV and temperature. Between sublingual and gastric microvascular variables, there was a positive correlation between sublingual MFI and gastric PPV. The remainder of the evaluated correlations was not statistically significant.

For investigator 1 (KD) the coefficient of variation was 5.04% (range 2.17-10.5) for TVD, 4.3% (range 2.6-6.4) for PVD, 1.9% (range 0-6.7) for PPV and 1.3% (range 0-10.2) for MFI. Less than 10% is generally considered to be acceptable. For investigator 2 (AD) the coefficient of variation was 14.5% (range 5.2-23.3) for TVD, 13.77% (range 3.7-23.3) for PVD, 2.35% (range 0-13.7) for PPV and 1.04% (range 0-5.6) for MFI. Interobserver variability, seen in Table 4, between KD and AD average microvascular variables was moderately strong, given that half of the calculated percent error were <30% and two additional were around 30%.
<table>
<thead>
<tr>
<th></th>
<th>Sublingual</th>
<th>Gastric</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables</strong></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Systemic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (per minute)</td>
<td>105 (85-179)</td>
<td>107 (70-176)</td>
<td>0.894</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>116 (87-183)</td>
<td>113 (100-138)</td>
<td>0.769</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>85 (64-113)</td>
<td>84 (71-110)</td>
<td>0.936</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>66 (48-94)</td>
<td>64 (51-88)</td>
<td>0.936</td>
</tr>
<tr>
<td>Temp(°F)</td>
<td>97.2 (94.3-100.4)</td>
<td>97.2 (94.3-100.2)</td>
<td>0.936</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>N/A</td>
<td>1.3 (0.6-2.4)</td>
<td>N/A</td>
</tr>
<tr>
<td>RR (per minute)</td>
<td>9 (6-16)</td>
<td>8 (5-16)</td>
<td>0.769</td>
</tr>
<tr>
<td>SpO\textsubscript{2} (%)</td>
<td>97 (92-99)</td>
<td>96 (94-99)</td>
<td>0.979</td>
</tr>
<tr>
<td>ETCO\textsubscript{2} (mmHg)</td>
<td>42 (20.5-49.3)</td>
<td>39.5 (22.4-48.5)</td>
<td>0.852</td>
</tr>
<tr>
<td><strong>Microcirculatory</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TVD (vessel/mm)</td>
<td>28.0 (21.9-31.3)</td>
<td>20.6 (14.5-28.7)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PVD (vessel/mm)</td>
<td>26.5 (20.7-31.2)</td>
<td>20.6 (12.9-28.5)</td>
<td>0.002*</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>96.8 (87.5-100)</td>
<td>100 (86.87-100)</td>
<td>0.101</td>
</tr>
<tr>
<td>MFI</td>
<td>3 (2.54-3)</td>
<td>3.0 (2.75-3.0)</td>
<td>0.139</td>
</tr>
</tbody>
</table>

Table 2 Averaged systemic variables and microcirculatory variables for the study population and p values obtained via Mann Whitney rank sum test. HR = heart rate, SAP = systolic arterial pressure, MAP = mean arterial pressure, DAP = diastolic blood pressure, Temp = temperature, TVD = total vessel density, PVD = perfused vessel density, PPV = proportion of perfused vessels, MFI = microvascular flow index, RR = respiratory rate, SpO\textsubscript{2} = pulse oximetry, ETCO\textsubscript{2} = end-tidal carbon dioxide, N/A indicates where data were not available. * Indicates p value <0.05
Table 3 Spearman’s rho correlations between the systemic variables and microcirculatory variables.  HR = Heart rate, RR = respiratory rate, SAP = systolic arterial pressure, MAP = mean arterial pressure, DAP = diastolic blood pressure, SpO\textsubscript{2} = pulse oximetry, EtCO\textsubscript{2} = end-tidal carbon dioxide, Temp = temperature, TVD = total vessel density, PVD = perfused vessel density, PPV = proportion of perfused vessels, MFI = microvascular flow index.  N/A designates data not available.  Those without statistical significance are reported as NS.  Those with statistical significance (at least p<0.05) are reported.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Bias</th>
<th>Limits of agreement</th>
<th>% error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sublingual TVD (vessels/mm)</td>
<td>30.00 ± 5.03</td>
<td>-4.92</td>
<td>-17.08 to 7.24</td>
<td>39.7%</td>
</tr>
<tr>
<td>Sublingual PVD (vessels/mm)</td>
<td>27.10 ± 4.66</td>
<td>-1.77</td>
<td>-11.51 to 7.98</td>
<td>35.2%</td>
</tr>
<tr>
<td>Sublingual PPV</td>
<td>90.54 ± 8.20</td>
<td>8.73</td>
<td>-3.61 to 21.07</td>
<td>13.4%</td>
</tr>
<tr>
<td>Sublingual MFI</td>
<td>2.80 ± 0.20</td>
<td>0.14</td>
<td>-0.32 to 0.60</td>
<td>16.1%</td>
</tr>
<tr>
<td>Gastric TVD (vessels/mm)</td>
<td>23.4 ± 6.09</td>
<td>-4.5</td>
<td>-15.04 to 6.04</td>
<td>44.4%</td>
</tr>
<tr>
<td>Gastric PVD (vessels/mm)</td>
<td>22.90 ± 6.40</td>
<td>-4.76</td>
<td>-15.34 to 5.82</td>
<td>45.3%</td>
</tr>
<tr>
<td>Gastric PPV</td>
<td>97.34 ± 4.51</td>
<td>-1.43</td>
<td>-10.17 to 7.31</td>
<td>8.8%</td>
</tr>
<tr>
<td>Gastric MFI</td>
<td>2.97 ± 0.10</td>
<td>-0.006</td>
<td>-0.31 to 0.29</td>
<td>8.6%</td>
</tr>
</tbody>
</table>

Table 4 Bland-Altman analysis evaluating interobserver variability between KD and AD for the average value for the 13 subjects. The mean ± SD is the mean of the combined average between investigators. The limits of agreement are two standard deviations from the differences between the two investigator averaged values. The % error is calculated by dividing $1.96 \times$ standard deviations from the bias by the combined group mean. TVD=total vessel density, PVD=perfused vessel density, PPV=proportion of perfused vessels, MFI=microcirculatory flow index.
Figure 11 Still image of a normal dog sublingual mucosal microcirculation with small arterioles, capillaries and venules outlined by erythrocytes which have absorbed the SDM light source.
Figure 12 Still image of the normal dog gastric serosal microcirculation with small arterioles, capillaries and venules outlined by erythrocytes which have absorbed the SDM light source.
Figure 13 Box-and-whisker plot for the statistically significant microvascular variables for both sublingual and gastric tissue beds. TVD=total vessel density, PVD=perfused vessel density

†Indicates significant difference between sublingual and gastric microcirculation parameters with p value <0.05
2.4 Discussion

The microcirculation is vital to distribution of blood and nutrients to tissues throughout the body. This study evaluated the microcirculation of two tissue beds, the sublingual mucosa and gastric serosa, of apparently healthy canines. It was determined that sublingual and aseptic intra-operative SDM imaging of a serosal surface is feasible and does not add significant surgical/anesthesia time. Gastric serosal and sublingual microvascular parameters for apparently healthy anesthetized dogs were also established. However, it was determined that, overall, correlation between sublingual and gastric mucosa microvascular variables and between systemic and microvascular variables was poor. Further, the intraobserver repeatability was variable with investigator 1 having strong repeatability and investigator 2 having only moderate. The interobserver variability was also found to be moderately strong with more than half of the variables compared via Bland-Altman and percent error being within or be approaching the acceptable range.

This study evaluated the use of the sublingual tissue for assessment of systemic microcirculation. In veterinary medicine, the buccal mucosa has been used most frequently, given there is potentially better opportunity to access this site in conscious patients. In addition, previous studies have involved patients in lateral recumbency. However, there are disadvantages to buccal mucosa, including interference from pigmentation and difficulty achieving good surface contact relative to conformation of the oral cavity especially when in dorsal recumbency. Both of these proved to be
significant issues in our patient population, resulting in the use of the sublingual site. This proved much easier to acquire quality microcirculatory videos, as previously utilized in a study evaluating the feline microcirculation. The sublingual tissue has been used frequently in the human clinical evaluation of various diseases, and has many advantages in humans, including ease of access and developmental association (as well as demonstrated correlation) with splanchnic circulation. When comparing our data to another veterinary study evaluating healthy canine buccal mucosa, the median values were very similar for all microcirculatory parameters. While the previous study had evaluated both the small and medium sized microvessels, our study evaluated only the microcirculation ‘small’ vessels measuring <20 um. Respectively the median values from our study compared to the median average of the three reviewers from the previous study: TVD 27.5 vessels/mm to 24.3 vessels/mm, PVD 26.22 vessels/mm to 24.3 vessels/mm, PPV 94.9% to 100%, and MFI 3.0 to 2.96. While a direct statistical comparison cannot be made, this suggests that in healthy anesthetized patients there are similarities in vessel density and perfusion between the two sites. And although every tissue bed will have different organizations of the capillary networks, given the similar embryological source, it is possible that these two tissue beds can be used interchangeably. Further studies comparing and correlating buccal and sublingual microcirculation would be beneficial to more definitively make this determination. Regardless, our study demonstrates that assessment of sublingual microcirculation can serve as a viable alternative to buccal mucosa. The most significant drawback is the need
for very heavy sedation or general anesthesia for placement under the tongue without risking damage to the probe.

Clinical and research use of SDM technology has surpassed previous tools for assessing the microcirculation. Although still early in its clinical use, future monitoring of patients may be possible, as demonstrated by studies that have successfully utilized this device over a prolonged time period.\textsuperscript{3, 6, 7, 27} Our group was able to obtain images of an intra-abdominal organ during a laparotomy procedure successfully. To maintain sterility, we covered the SDM hand-held tool with a sterile long palpating sleeve and a specially designed sterile probe cover. Early on it was found that abrupt temperature change from the ambient room to the tissue temperature resulted in fogging of the SDM probe end leading to loss of image quality (primarily poor focus). To minimize this impact, the tool was kept within the axilla of an investigator and a drop of anti-fogging solution (commonly utilized in laparoscopic procedures) was applied to the probe end prior to connecting the sterile probe cover. This served to significantly improve image quality. During video capture of the gastric tissue, we recognized difficulty in obtaining the ideal 20 second long stable images, primarily through gastric serosa movement during respirations, and peristalsis. When respirations were a significant interference, it was determined that placing the patient on a mechanical ventilator facilitated the ability to control ventilation and improve the possibility of stable video capture. Further adjustments may be needed for future studies utilizing this technology on other visceral organs.
To facilitate utility of bed side use, additional scoring systems have been developed in both human and veterinary medicine but the use of these scoring systems requires further validation.28,29 In this study, we achieved 93% of the desired number of videos for analysis among the fifteen patients, indicating good success of obtaining quality images for data compilation. Further, inadequate videos were confined to the gastric location of two patients, suggesting there may have been factors specific to those circumstances (excessive peristalsis, hyperventilation, etc) rather than the technique itself. We also found that time needed to obtain these images is relatively small, an average of 5 minutes at each site. While there was no significant difference for sublingual video capture between early and late groups, there was significant improvement for gastric video acquisition time (from approximately 6.5 to 3.5 minutes). This suggests that while there was a steeper learning curve for acquiring quality gastric videos, ultimately they could be obtained (on average) faster than at the sublingual site. Given the desire to limit anesthesia time, this also supports the notion that intra-operative microvascular assessment can be performed with minimal impact.

An additional concern for this technology as a bed side tool is intraobserver and interobserver variability. Our study supports good agreement both within and between observers, which has been corroborated in previous studies.29,30 The repeatability of investigator 1 (KD) was excellent, with all coefficient of variation <10% (which is the generally accepted cutoff of acceptability).23 However the overall repeatability of investigator 2 (AD) was only moderate, with half of the coefficient of variation >10%.
For calculation of the intraobserver variability, the eight random videos were analyzed for the second and third time following completion of all video analysis. Therefore, some of the intraobserver variability may have reflected a change in skill level from the first analysis. When considering the interobserver variability, the variability was only moderate, given that approximately half of the calculated percent error for the two investigators was <30%, previously described as acceptable error for repeatability.\textsuperscript{25} Both video analyzers underwent a similar training period with an experienced investigator but overall were new to performing vascular analysis. This demonstrates that although there may be a steep learning curve to analyzing the videos, there is high feasibility of this tool in clinical use is highly possible.

Short of intraoperative assessment during laparotomy, direct microcirculatory assess of the stomach or other visceral organs is not possible with SDM. As such, a major goal of this study was comparison of sublingual and gastric serosal microcirculation to assess whether the former (being more readily accessible) can be used as a surrogate. With this study it was determined that there is a significant difference for TVD and PVD values (Figure 11), while no difference was found with PPV or MFI. When evaluating the correlation of microcirculatory variables between the two sites, the only statistically significant correlation was between the sublingual MFI and gastric PPV. These combined statistical results suggest that overall vessel density is less in gastric serosa compared to sublingual, that optimal perfusion and homogeneity of blood is maintained in both tissues, but there is poor correlation (i.e. that changes in one cannot necessarily be
used to predict changes in the other). This is in contrast to previous studies of sublingual circulation in human patients which have reported good correlation to the gastrointestinal microcirculation.\textsuperscript{1,26} There are several potential explanations for these findings. It is possible there could have been an alteration in microcirculation associated with manipulation of gastric tissues during video acquisition or because of a decrease in surface temperature from atmospheric exposure during laparotomy. Every effort was made to minimize these effects but they certainly could have impacted vessel density, as well as correlation with sublingual tissues. The inability to find correlation of microvascular variables between sites may also be explained by the low number of subjects, even though a priori power calculation (suggesting 12 subjects would be sufficient) was surpassed. Further, given a low subject number, it was decided to use non-parametric tests (even though microcirculatory data was normally distributed). This may also have served to make it more challenging to find a significant correlation. Therefore, additional studies with more subjects may elucidate both predictive value and correlation between sublingual and gastric microcirculation.

One of the challenges in trying to assess microcirculatory changes is the ability to gain access to the vascular beds of interest. Systemic variable parameters, such as mean arterial blood pressure, cardiac output and heart rate, can be more easily obtained in clinical patients. As such, the hope would be to use these variables as a surrogate to reflect changes in the microcirculation. Unfortunately, many studies, including the current study, have failed to find a relationship between macro and microcirculation,
especially in disease states. Olofsson et al found, when evaluating the gastric perfusion specifically, that the mean arterial pressure did not change even when a concurrent decrease in gastric perfusion was documented. The overall poor correlation to systemic variable parameters found in the current study may be due to the evaluated subjects being apparently healthy. These patients were unlikely to have lost either local autoregulation or the inability to maintain systemic blood pressure. Evaluation of these same tissue beds in a disease state may yield completely different results. There was a negative correlation found between the respiratory rate and the sublingual PVD and PPV and a negative correlation between the end-tidal carbon dioxide levels and the gastric TVD. It is possible that this may reflect the impact of carbon dioxide levels on vascular tone within the microcirculation. When elevated, carbon dioxide will cause vasodilation; therefore in this study those patients with the lower respiratory rate may have concurrently had elevation in arterial carbon dioxide. This would then lead to an increase in the PVD and PPV in response to vasodilation. However, this is not supported by the negative correlation for sublingual or gastric microcirculation with EtCO$_2$. This suggests that increased EtCO$_2$ (and presumably arterial CO$_2$) was associated with a decrease in sublingual TVD and gastric TVD and PVD, not the expected increase that should come from increasing arterial CO$_2$ and vasodilation. Interestingly, a recent study evaluating the sublingual microcirculation of cats failed to find a correlation with any respiratory variables, including EtCO$_2$, SpO$_2$, and respiratory rate. However, in the absence of direct measurement of PaCO$_2$ it is difficult to know if this relationship truly exists. Additionally there was a negative
correlation between the gastric TVD and PVD compared to pulse oximetry. This is interesting given the expected impact of arterial oxygen tension on vascular tone is a positive correlation. The cause of this contradictory finding is unclear, as is the impact of the findings on the results. Further evaluation, including arterial oxygen and carbon dioxide levels is warranted in future studies. Finally, there was a negative correlation for temperature with sublingual PPV and gastric MFI. This is perhaps the opposite of what was expected given an expected vasoconstriction with hypothermia. This may continue to support the local regulation ensuring persistence of microvascular perfusion even in the face of decreased temperature. Interpretation of these results must also take into account that microcirculatory images were obtained from two tissue beds that are normally exposed to different environmental temperature. The exposure of the gastric tissues to ambient temperature, leading to a tissue temperature that is not normal body temperature, may lead to a reflexive local vasoconstriction of the mucosal or serosal surface. Therefore, the intra-esophageal temperature may not be reflective of the tissue bed temperature and therefore may be the reason that the correlation with temperature was not what would otherwise be expected (i.e negative instead of positive). The direct temperature of the tissue was not obtained at the time of the study, but may be a future avenue of further investigation.

A limitation to this study is the requirement for anesthesia which may affect the microcirculation. The impact of anesthetic agents on the microcirculation may include diffuse vasodilation secondary to anesthesia inhalants.\textsuperscript{34,35} Therefore, it is possible that
the general anesthesia may impact the microcirculation in this population of animals. This may be avoided in disease states by limiting the site of evaluation to the sublingual tissue. Further influence of anesthesia in this population was the potential impact of using two different anesthesia induction protocols, although this was not initially a goal of the study. One subset of patients received ketamine in addition to propofol (n=5), while the other received only propofol. Somewhat surprisingly, there were no statistically significant differences between these groups for either systemic or microcirculatory variables. Based upon the pharmacological impact of these drugs, some change had been expected. Ketamine, via increased sympathetic tone, can cause an increase in cardiac output, increased heart rate, and increased mean arterial pressure, although the effects on the total peripheral resistance can vary.\textsuperscript{36} In contrast, propofol, may lead to hypotension, bradycardia, and negative inotropism.\textsuperscript{36} Previous studies have evaluated the microcirculatory impact of various anesthetic induction protocols (including ketamine) with varying results. Enouri et al, found an increased heart rate, MAP, cardiac output, and cardiac index for those patients receiving a ketamine-diazepam regimen compared to propofol and thiopental alone.\textsuperscript{37} Riccó et al found that ketamine-propofol induction resulted in a significantly lower SAP and MAP compared to baseline, however no major differences between the induction groups was found for any variable.\textsuperscript{38} Another study found that hemodynamic effects were similar between all treatment groups, although the stroke index for propofol-lidocaine-ketamine group was lower than the propofol alone group.\textsuperscript{39} Previous studies have evaluated the impact of ketamine on the microcirculation with varying results. One study did not find any alteration of the
functional capillary density (FCD), equated to PVD in the present study, on intestinal microcirculation in anesthetized endotoxemia-induced rats. Another study found that the FCD of rat ileal microcirculation to be decreased when anesthetized with ketamine and midazolam. A third study found vasoconstriction within the skeletal microcirculation when ketamine was used. Regardless of these previous findings and study impacts, the current study did not find any significant differences between the two induction protocols which may suggests a minimal impact of ketamine on the systemic or microcirculatory variables in our patient population.

Although our study has established evaluation of the normal canine gastric serosa and the sublingual microcirculation, there are several limitations. One such limitation may include the use of the SDM technology, which has its own inherent limitations. This technology can only be used on mucosal or serosal surfaces, such as the sublingual tissue or gastric serosa, limiting its utility elsewhere (such as cutaneous epithelium). Gastric peristalsis and patient ventilation limited the duration of image capture without significant motion artifact, thereby shortening the window for assessment to only 5 seconds (instead of the recommended 20 seconds). This short time period may alter impressions of vascular perfusion and would likely lead to an over-estimation of the perfusion. With a shorter period of observation, intermittent flow may be misinterpreted as continuous and therefore incorrectly labeled as perfused. Microcirculation within tissues may have heterogeneity of flow, especially in disease states, making the timing of video stability a risk for incorrect assessment of the tissue bed overall perfusion. Final
modality limitations include the delay in image analysis which may make this technology difficult to evaluate disease states especially in the clinical setting. Another limitation is the low number of subjects. Although power was calculated and reached, additional subjects may expand upon the findings of this paper. Further, two subjects had to be removed for lack of quality gastric videos.

2.5 Conclusion

This study established microvascular variables for sublingual and gastric serosa in apparently healthy patients undergoing elective gastropexy. Further, it served to demonstrate a technique for intra-operative assessment of serosal microcirculation of abdominal viscera. Our study did not find correlation between the sublingual microvasculature to the gastric microvasculature. Additional studies should be performed, especially evaluating the gastric microcirculation in disease states.

2.6 Footnotes
a = Acepromazine, Butler Schein Animal Health, Dublin, OH.

b = Morphine sulfate, West ward, Eatontown, NJ.

c = Hydromorphone hydrochloride, West ward, Eatontown, NJ.

d = Methadone, Mylan Laboratories, Canonsburg, PA.

e = PropoFlo, Abbott Laboratories, North Chicago, IL.

f = Ketamine hydrochloride, Pfizer, Inc., New York, NY.

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g = Fluriso, MWI, Boise, ID.

h = Cardell® MAX-12 DUO HD Multiparameter Monitor, Midmark Corp, Versailles, OH.

i = Lactated Ringer’s Solution, Baxter Healthcare, Deerfield, Ill.

j = Model 811-B Doppler Ultrasound, Parks Medical Electronics, Inc., Aloha, OR.

k = Lactate Plus, Nova Biomedical Corporation, Waltham, MA

l = Microscan, MicroVision Medical, Amsterdam, The Netherlands.

m = Ag-Tek® MaxiSleeve®, Neogen® Corporation, Lexington, KY.

n = MediChoice® Endoscopic Anti-Fog Solution, Owens & Minor, Richmond, VA.

o = Sofsilk®, Covidien Ltd., Dublin, Ireland.

p = PDS™ II, Ethicon, Inc, Cincinnati, OH.


r = IBM® SPSS Statistics Version 21

2.7 References


Chapter 3: Microcirculation in Gastric Dilatation Volvulus

3.1 Introduction

Gastric dilatation volvulus (GDV) is a common disease, with a reported occurrence of 2.4 to 7.6 dogs per 1000 admission.\(^1\) Although several risk factors have been identified, the etiology remains unknown. Without surgical intervention, the disease process itself is fatal. However, every patient is also at risk for severe secondary complications including tissue hypoxia, gastric necrosis, systemic inflammatory response syndrome, sepsis, peritonitis, disseminated intravascular coagulopathy and death.\(^2-4\) Several studies have shown a variable mortality rate, reported as 10% to 33%.\(^1,5-8\) Risk factors for death have been identified and include duration of pre-admission clinical signs, development of acute renal failure, need for splenectomy, presence of gastric wall necrosis and untreated gastric wall necrosis.\(^2,9\) Gastric wall necrosis in particular has been found in 10% to 25% of cases,\(^6-8,10,11\) and has been associated with increased risk of secondary complications including multiple haemostatic abnormalities\(^12\) and increased mortality rate.\(^5-7,9,12,13\) One study found macroscopic gastric wall necrosis in 19% of the GDV patients, 62.5% of which did not survive to discharge and all dogs that died had gastric necrosis.\(^14\) Despite advancements in medical and surgical management, the mortality rate, especially when associated with gastric necrosis, remains significant.
Currently, intra-operative evaluation of gastric necrosis is subjective and based on tissue thickness and texture, surface color, presence of peristalsis, and serosal capillary perfusion. However, this subjective assessment of the gastric wall may not accurately reflect tissue viability, especially at the microcirculatory level. It has been reported that misinterpretation can occur in 40% of cases leading to incomplete removal of compromised or nonviable gastric wall with increased risk of stomach rupture or surgical dehiscence. Further, serosal changes may not be reflective of mucosal ischemia, and therefore necrosis may be more extensive than can be externally assessed during surgery. As such, the ability to perform more direct intra-operative assessment of the microcirculation may allow for better determination of gastric blood flow and viability.

The microcirculation consists of the arterioles, capillaries and venules (< 200μm diameter), and is responsible for distribution of blood flow to organs and therefore is vital for maintaining tissue perfusion. In health, there is local tissue autoregulation which maintains adequate microcirculatory blood flow in the face of systemic compromise. However, this autoregulation may be lost in an assortment of diseases, leading to a loss of blood flow and tissue perfusion. Therefore, systemic variable changes (heart rate, blood pressure, cardiac output, lactate, etc) may not fully demonstrate this loss of microvascular supply. This potential for disconnect between macrovascular flow and microvascular perfusion has led to the development of techniques to allow for more direct evaluation of the microcirculation.
One such technology is sidestream dark field microscopy (SDM). With SDM, a polarized green light with a wavelength of 530 nm is directed onto tissue surfaces and reflected.\textsuperscript{19,20} The hemoglobin of erythrocytes within the tissue absorbs the light, allowing for 326x magnification and real-time visualization of flow through the microcirculation.\textsuperscript{19,20} Further, SDM allows for off-line evaluation of several microcirculatory components based on previously established consensus criteria. Total vessel density (TVD) is the total vessel density within the imaged field\textsuperscript{19} whereas proportion of perfused vessel (PPV) reflects the proportion of these vessels that have blood flow.\textsuperscript{21} Perfused vessel density (PVD) is the functional density of vessels available for perfusion and the heterogeneity of perfusion.\textsuperscript{22} The microvascular flow index (MFI) represents the semi-quantitative assessment of erythrocyte movement and flow within the vessel.\textsuperscript{22} These combined features allow the clinician to determine the quantity and quality of the microcirculatory blood flow.

Assessment with SDM has shown both clinical and experimental relevance in humans.\textsuperscript{23-27} With this technology, microvascular alterations within the microcirculation have been implicated in multiple organ failure\textsuperscript{18} and as an indication of post-operative complications.\textsuperscript{28} In a study of septic shock, recovery of microcirculatory alterations within the first 24 hours was indicative of survival.\textsuperscript{29} Evaluation of the microcirculation has also emerged as a new means of assessing target organ perfusion during resuscitation.\textsuperscript{30-33} Additionally, and possibly relevant to GDV, human studies \textsuperscript{34,35} have found that an increase in intra-abdominal pressure is positively correlated with the
impairment of gastric serosal microcirculation. Furthermore, studies have also established a good correlation between gastrointestinal and sublingual tissues, making this a possible surrogate for disease of the gastrointestinal tract.

Veterinary medicine has begun to evaluate the microcirculation in health and disease. One study evaluated the buccal mucosal circulation in healthy dogs, while other studies utilized intestinal microcirculation in hemorrhagic shock and during infusion of a hyperviscous solution. Although many human studies have utilized the sublingual tissue microcirculation in disease, buccal mucosa is the primary site utilized up to this point in veterinary medicine. One recent study has evaluated the sublingual tissue in cats, with good success. While previous literature has established the feasibility of SDM in veterinary medicine, there is a lack of specific research in microcirculatory changes in gastric dilatation volvulus.

The goal of this study was to evaluate the gastric serosal microcirculation before and after derotation in naturally occurring GDV patients as compared to healthy control patients undergoing elective gastropexy. Secondary goals were comparison and determination of correlation between the gastric serosa microcirculation to the sublingual microcirculation and between microvascular parameters and systemic variables. We hypothesized that the gastric serosal microcirculation would be compromised in GDV (compared to control) but would have improvement after derotation, that there would be good correlation between gastric serosal and sublingual microcirculation, and that there would be poor correlation between microcirculation variables and systemic variables.
3.2 Material and Methods

3.2.1 Animals

The study protocol was approved by The Ohio State University Institutional Animal Care and Use Committee. Canine patients with naturally occurring gastric dilatation volvulus (GDV) confirmed via right lateral radiograph, presenting to The Ohio State University were prospectively enrolled with informed client consent obtained prior to enrollment. Pre-operative patient management through the Emergency Service, including fluid resuscitation and other stabilization measures (i.e. trocharization), and additional diagnostics (i.e. blood work, additional radiographs), was at the discretion of the primary clinician. Exclusion criteria included those patients deemed too unstable for the additional anesthesia time needed for intra-operative data collection, if client did not consent to surgery, or if client did not consent for enrollment in the study.

3.2.2 General anesthesia, instrumentation and surgical approach

All patients were placed under general anesthesia with a protocol chosen at the discretion of the anesthesia technicians and/or anesthesiologist based on the clinical status of the patient. The premedication protocol consisted of hydromorphone\(^c\) (doses from 0.05mg/kg to 0.1mg/kg) or methadone\(^d\) (doses from 0.2mg/kg and 0.3mg/kg) and midazolam\(^a\) (doses from 0.12mg/kg to 0.2mg/kg). Anesthesia induction was achieved with propofol\(^e\) or propofol\(^f\) and ketamine\(^f\) (doses from 0.95mg/kg to 4mg/kg and 1.4mg/kg respectively). Anesthesia was maintained via an inhalant\(^g\) on 100% oxygen and fentanyl\(^a\) constant rate infusion (at 5 mcg/kg/hr). All patients had at least one
peripheral intravenous catheter placed at the time of presentation as well as a central catheter placed through the jugular vein for central venous blood sample collections. Placement of an arterial catheter in the dorsal metatarsal artery was performed at the discretion of the anesthesia staff as needed for direct blood pressure monitoring. All patients received intravenous crystalloid fluid and/or colloid fluid therapy at the discretion of the anesthetist.

After routine surgical preparation for an abdominal exploratory surgery, all patients were transported to the surgical suite and placed in dorsal recumbency. All patients were connected to a hemodynamic monitor, as described below, and were provided standard surgical thermal support as needed. The patient was draped as deemed appropriate for standard mid-line exploratory laparotomy approach into the abdomen. At the time of initial abdominal explore, the decision to pass an orogastric tube or intraoperative trocharization for decompression was at the discretion of the surgeon.

3.2.3 Measurement of systemic variable parameters

Admission data collected for all patients included heart rate (HR), respiratory rate (RR), arterial blood pressure (systolic or systolic, diastolic and mean) and peripheral lactate. After admission and initial resuscitation but prior to anesthesia, the following data was obtained if possible: heart rate, blood pressure (systolic or systolic, diastolic and mean), and pulse oximetry. A peripheral lactate was obtained in cases as deemed necessary by the attending clinician at varying post-resuscitation time points.
Time points for systemic variable data collection were established relative to gastric position and tissue location: pre-derotation sublingual mucosa (PrS), pre-derotation gastric serosa (PrG), post-derotation sublingual mucosa (PtS) and post-derotation gastric serosa (PtG). At each time point, systemic variable data obtained, including heart rate and blood pressure\textsuperscript{h} (systolic, diastolic and mean) via direct or oscillometric methods, and respiratory variables of respiratory rate\textsuperscript{h}, pulse oximetry\textsuperscript{h} and end-tidal carbon dioxide\textsuperscript{h} (EtCO\textsubscript{2}). Immediately upon completion of gastric serosal videos (PtG), a central venous sample\textsuperscript{x,y} was obtained to determine lactate and central venous oxygen saturation (ScvO\textsubscript{2}). Final data was obtained post-operatively in anesthetic recovery (Rec) including heart rate, blood pressure\textsuperscript{i}, respiratory rate, and pulse-oximetry\textsuperscript{i}. Patients recovered in the intensive care unit with post-operative care at the discretion of the attending clinician.

3.2.4 Microcirculatory video capture

SDM videos were obtained at the following time points for the specific tissue beds (if possible): prior to induction (PrI) buccal, pre-derotation sublingual (PrS), pre-derotation gastric (PrG), post-derotation sublingual (PtS), post-derotation gastric (PtG), and recovery (Rec).

All videos were acquired by a single investigator (ESC) with an SDM system\textsuperscript{l} and using a specifically designed probe cover (Figure 7). The Microscan unit was connected to a personal laptop and monitor, allowing for real-time video display. At the time of acquisition, videos were assessed for clarity, stability and evidence of compression.
artifact.\textsuperscript{22, 43} Adjustments were performed to ensure optimum image quality, as previously described.\textsuperscript{43} Based upon previous established consensus criteria,\textsuperscript{22} at least three videos of 20 second duration at each time point and each tissue bed is recommended for optimal analysis. If there was question regarding quality, such as focus, vessel compression, significant movement, or fluid/blood obscured the field, additional videos were obtained. Videos were acquired until it was believed that at least three quality videos of 20 second duration were obtained at each tissue bed for each time point. The number of videos acquired and total time to obtain videos was recorded.

\textit{Buccal mucosa}

Prior to induction, the SDM probe was lightly placed against the buccal mucosa at the mucogingival junction above the carnassials tooth and balanced upon the table or bedding to minimize motion (figure 14). Prior to induction it was necessary to use this site because the patients were not sedate enough for access to the sublingual location with risk of damage to the probe or injury to the investigator. Optimal probe contact (based upon image quality) was ensured via gentle removal of excessive saliva or application of saline. If pigmentation was present, the microscan unit was moved to the nearest area of buccal mucosa that was not pigmented. If there were no areas without pigment, if patient compliance did not allow application of the SDM probe, or the patient had already been transported to the anesthesia preparation area prior to investigator arrival, videos at this time point and location were not obtained.
Sublingual mucosa

Sublingual videos were obtained prior to gastric derotation (PrS), after gastric derotation (PtS) and in anesthetic recovery (Rec). In previous reports of SDM use in dogs, the buccal mucosa has been used for assessment of the microcirculation. However, in pilot investigations, it was determined that patient positioning during anesthesia (dorsal recumbency) and tissue pigmentation made it difficult to consistently obtain quality buccal images. As such, it was decided to instead examine the sublingual mucosa, given that this area has been utilized in human medicine and successfully explored in feline patients. In order to minimize the impact on anesthesia time, and allow adequate time for the investigator to perform surgical scrub prior to intra-abdominal imaging, PrS video were obtained during final surgical prep and draping, and just prior to or during the initial laparotomy incision. The PtS videos were obtained immediately after the PtG videos. To obtain sublingual images, the microscan probe was lightly placed against the base of the dog’s frenulum and braced against the table to minimize motion (Figure 8). Optimal probe contact (based upon image quality) was ensured via gentle removal of excessive saliva or application of saline.

Gastric serosa

To maintain intra-operative sterility while obtaining videos, one investigator performed routine procedures for surgical scrub, gowning and gloving. A long sterile palpation sleeve was used to cover the SDM hand-piece and the proximal portion of the
data cable at risk of contact with the surgical field. In addition, the probe itself was covered with a new sterile cover (Figure 7). During preliminary attempts, it was discovered that significant condensation accumulated between the probe and the cover resulting in diminished image quality. It was suspected to be due to the change of the probe surface from room temperature to body temperature. To limit this effect, the probe was pre-warmed in the axilla of a co-investigator just prior to intraoperative use and an anti-fogging solution was applied to the probe prior to attachment of the sterile cover.

The gastric serosa videos were obtained from a consistent location on the fundus toward the greater curvatures of the stomach (Figure 10), even if there appeared to be areas of decreased gastric viability in other portions of the stomach. For the videos prior to derotation, a small rent within the mesentery was made to allow access to the gastric serosa. This was done prior to any gastric tissue manipulation by the surgeons. However, if deemed necessary the stomach may have been decompressed by passage or an orogastric tube or trocharization prior to obtaining these videos. If the patient’s stomach had derotated without surgeon intervention, these videos were not obtained. Once video acquisition was completed, the stomach was de-rotated and the remainder of the abdominal exploration was performed. The PtG videos were then obtained (approximately 10-15 minutes after PrG) from a consistent location on the fundus toward the greater curvature of the stomach (Figure 10) just before the gastropexy was performed. Any additional procedures, such as splenectomy or gastric resection, were then performed as deemed necessary by the surgeon.
Any blood pooling in the area was light blotted away with a moistened gauze. During video capture for PtG, while the majority of the parameters used to assess image quality were considered satisfactory, all contained some motion from peristalsis and/or ventilation. If after 10 minutes it was not possible to acquire suitable videos, efforts were discontinued. At both gastric time points, surgeons used previously established convention\textsuperscript{5, 10, 14, 15} to assess overall gastric tissue viability. In order to minimize bias, this information was not expressed to the investigators until the patient had recovered from anesthesia.

Figure 14 Representative picture demonstrating buccal mucosal microcirculation image contact for SDM video capture.
3.2.5 Microcirculatory video analysis

After completion of video capture, the videos were analyzed for quality (image resolution, motion, and the presence of pressure artifact) by one investigator (ESC). During this assessment it was determined that motion created by peristalsis and ventilation precluded the ability to obtain steady gastric serosa videos for the recommended 20 seconds. The longest consistent period without motion was 5 seconds. As such, the videos were trimmed to that time frame. If quality videos of at least 5 seconds duration could not be obtained, the videos were withdrawn from analysis. The three videos of best quality from each subject/location were renamed, randomized and moved forward to vascular analysis. Vascular analysis was performed to determine microcirculatory variables (TVD, PPV, PVD, and MFI) using software specifically designed for use with SDM. Detailed descriptions of video analysis and how these values are determined can be found in numerous sources. A separate investigator (AD) analyzed the screened videos and was unaware of the patient identity, video capture location, or the time point.

3.2.6 Statistical analysis

The data were evaluated for normality graphically and with the Kolmogorov-Smirnov and Shapiro-Wilk tests. Descriptive statistics were computed for all data and reported as median (range) to allow for consistent data reported, even if the data was normally distributed. Given the low number of patients and a mix of data that was both normally and not normally distributed, it was elected to perform all statistical
examinations with non-parametric tests. Time-point specific statistical analysis for systemic and microcirculation variables between GDV and previously established data from control dogs were conducted using Mann-Whitney U test. Comparison between the pre-derotation and post-derotation gastric microcirculation variables was performed with Wilcoxon signed rank test for related samples. Comparison of each sublingual microcirculation variable across the four time points was performed with a Friedman’s two-way ANOVA for related samples. For each time point, Spearman’s rank correlation was used to identify correlations between 1) the independent systemic variable values and the microvascular data and 2) between sublingual and gastric microvascular data at pre-derotation and post-derotation.

The statistical analysis was performed with commercially available computer software. For all analyses, p < 0.05 was considered significant. Power was calculated a priori as needing 12 dogs based upon previously reported studies.19,39

3.3 Results

A total of 13 dogs were prospectively enrolled. One patient was excluded after enrollment due to the ability to only obtain data at only half of the desired time points. Therefore 12 patients were included for analysis. The average age of the patient population was 8.07 years (±3.84) with an average weight of 37.1 kg (±6.40). The dog breeds included mixed breed dog (n=3) and one each Akita, Great Dane, Gordon Setter, German Shorthair Pointer, Borzoi, Labradoodle, Golden Retriever, Labrador Retriever and German Shepherd. All patients had at minimum a packed cell volume and total
solids performed, with a mean of 47.3% (±8.36) and 6.92g/dL (±1.80) respectively. Admission lactate was obtained for all patients with a median of 3.5mmol/L (0.7-13.2). Trocherization was performed in five dogs prior to surgery.

Intraoperatively, all surgeons demonstrated concern about gastric tissue health at evaluation prior to derotation. However, all but one patient’s subjective assessment of gastric tissue health had markedly improved upon re-examination after derotation and abdominal explore. One patient required gastric resection in the fundic region of the stomach as well as a splenectomy (the spleen was subsequently diagnosed as having a round cell malignancy). Additional procedures performed included a gastrotomy for removal of gastric foreign material (n=1) and a liver lobe biopsy of one patient (diagnosed as having hemangiosarcoma). All patients survived to hospital discharge.

A total of 296 videos were obtained with 30 videos of buccal, 160 videos of sublingual, and 106 videos of gastric tissue. The total time to acquire videos was 217 minutes, with 19 minutes for buccal mucosa (0.63 minutes per video), 120 minutes for sublingual videos (0.75 minutes per video), and 78 minutes for the gastric tissues (0.74 minutes per video). Time to acquire the buccal mucosal videos per patient was 2.7 minutes (n=7). Total time to acquire all time point sublingual videos per patient was 10 minutes (n=12) When considering time of video acquisition of the gastric tissue, the average time per patient was 6.5 minutes (n=12).
Not all patients had three videos of sufficient quality or videos were not obtainable. For PrI, 5/12 patients were not included due to an inability to obtain buccal videos, most commonly because of poor patient compliance and the presence of tissue pigmentation. Quality sublingual videos were obtained for all subjects at PrS, PtS, and Rec. For gastric video acquisition, acceptable videos could not be obtained for 6/12 PrG and 1 patient at PtG. Most common reasons were inability to achieve adequately focus, blood pooling and/or gastric peristalsis. Representative images of the microcirculation at each time point are shown below as Figures 15.

Systemic variables obtained at the previously described time points are summarized in Table 5. The median (range) of the microvascular variables at the specified time points are located in Table 6. Previously established sublingual and gastric microcirculation values in healthy anesthetized patients (control) were compared to those from this GDV population at each available time point (pre and post-derotation). Further, systemic variables between the two groups were also compared at each time point. For this comparison, the only significantly different values were found at pre-induction for RR (9 vs 20, p=0.009) and systolic blood pressure (116 vs 132 mm Hg, p=0.005), and at the time of recovery RR (9 vs 16, p=0.004) (Table 5). All other control systemic variables were not significant different. Microcirculatory comparisons between GDV and control revealed a number of significant differences, with an increase in TVD at all time points, but decreased in PPV and MFI at PrS and PtS for sublingual (Table 6). All microcirculatory values were decreased for PrG compared to control, whereas only TVD
and PVD were decreased after derotation (Table 6). These comparisons are further represented in graphic form (Figures 16 to 23).

When microvascular changes were assessed across time points within the gastric microvascular variables, there were significant differences in PPV (p=0.028) and MFI (p=0.043) after derotation (Table 6). When changes were assessed across time points for buccal and sublingual mucosal microvascular variables no significant differences were found (Table 6). Comparison between sublingual and gastric microvascular at the pre-derotational time point revealed a significant positive correlation with MFI (r=0.829, p=0.042). At the post-derotational time point, PVD approached having a significant negative correlation between the two sites (r=-0.600, p=0.051).

Correlations between the systemic variables and microvascular parameters at the respective time points were evaluated. At the PrS time period, there was a negative correlation between PPV and HR (r=-0.704, p=0.011), and positive correlation for MFI and EtCO₂ (r=0.592, p=0.043) and the PPV to ScvO₂ (r=0.609, p=0.047). There was a positive correlation at PrG between PVD and SpO₂ (r=0.853, p=0.031). At the PtS time point, there were positive correlations between TVD and lactate (r=0.774, p=0.003) and PVD to lactate (r=0.718, p=0.009). At PtG, there were negative correlations between TVD and HR (r =-0.664, p=0.026) and MFI to lactate (r=-0.740, p=0.009). Finally in Rec period, there was a negative correlation between the RR and MFI (r=-0.588, p=0.044).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Adm</th>
<th>PrI</th>
<th>PrS</th>
<th>PrG</th>
<th>PtS</th>
<th>PtG</th>
<th>Rec</th>
</tr>
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<tbody>
<tr>
<td>HR (beats per minute)</td>
<td>105 (85-179)</td>
<td>161</td>
<td>120</td>
<td>127.0 (80-182)</td>
<td>118 (70-165)</td>
<td>120.0 (85-154)</td>
<td>125.5 (87-150)</td>
<td>110.0 (84-140)</td>
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<tr>
<td>RR (breaths per minute)</td>
<td>9 (6-16)</td>
<td>X</td>
<td>20 (12-36)*</td>
<td>10.5 (8-40)</td>
<td>11.5 (8-40)</td>
<td>10 (5-18)</td>
<td>10.5 (6-19)</td>
<td>16.0 (8-32)*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>116 (87-138)</td>
<td>141</td>
<td>132</td>
<td>114.5 (84-143)</td>
<td>114.0 (95-145)</td>
<td>119 (102-145)</td>
<td>115 (84-142)</td>
<td>116.0 (92-190)</td>
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</tbody>
</table>

Table 5 Patient systemic variables at the designated time points and tissue location. All data points are presented as median (range). Adm = admission, PrI = pre-induction buccal, PrS = pre-derotation sublingual, PrG = pre-derotation gastric, PtS = post-derotation sublingual, PtG = post-derotation gastric, Rec = recovery, HR = heart rate, RR = respiratory rate, BP = blood pressure, SpO2 = pulse oximetry, EtCO2 = end tidal carbon dioxide, ScvO2 = central venous oxygen saturation, x = indicates data not available either because not collected or too few patient number for analysis. * = indicates significantly different compared to control at p <0.05.
<table>
<thead>
<tr>
<th></th>
<th>Mean BP (mmHg)</th>
<th>Diastolic BP (mmHg)</th>
<th>SpO₂ (%)</th>
<th>EtCO₂</th>
<th>Lactate (mmol/L)</th>
<th>ScvO₂ (mmHg)</th>
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<tbody>
<tr>
<td></td>
<td>85 (64-113)</td>
<td>121 (105-146)</td>
<td>97 (92-99)</td>
<td>42 (20.5-49.3)</td>
<td>1.3 (0.6-2.4)</td>
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<td>93.5 (80-145)</td>
<td>102 (60-127)</td>
<td>97.5 (94-98)</td>
<td>X</td>
<td>3.5 (0.7-13.2)</td>
<td>X</td>
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<td></td>
<td>79.5 (62-100)</td>
<td>64.5 (51-125)</td>
<td>X</td>
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<td>81 (65-105)</td>
<td>60.5 (47-84)</td>
<td>95.5 (92-99)</td>
<td>39 (19-53)</td>
<td>1.4 (0.3-5)</td>
<td>X</td>
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<td></td>
<td>84.0 (65-106)</td>
<td>63.5 (45-94)</td>
<td>96 (90-98)</td>
<td>41.5 (20-53)</td>
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<td>83.5 (62-107)</td>
<td>68.5 (53-99)</td>
<td>96 (91-98)</td>
<td>35 (20-45)</td>
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<td>70.5 (54-77)</td>
<td>96 (93-97)</td>
<td>40.5 (20-48)</td>
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<td>95 (90-99)</td>
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</tr>
<tr>
<td>Variable</td>
<td>Control</td>
<td>Pre-induction</td>
<td>Prederotate</td>
<td>Postderotate</td>
<td>Recovery</td>
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<tr>
<td><strong>Buccal/Sublingual</strong></td>
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<td></td>
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<tr>
<td>TVD</td>
<td>28.0 (21.9-31.3)</td>
<td>32.7 (28-40)*</td>
<td>32.5 (27-51)*</td>
<td>31.6 (28-42)*</td>
<td>35.4 (23-41)*</td>
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<tr>
<td>PVD</td>
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<td>29.9 (13-35)</td>
<td>27.9 (18-41)</td>
<td>29.9 (23-38)</td>
<td>33.3 (18-37)</td>
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<tr>
<td>PPV</td>
<td>96.8 (87.5-100)</td>
<td>91.0 (44-99)</td>
<td>88.3 (62-97)*</td>
<td>90.8 (76-97)*</td>
<td>91.4 (63-99)</td>
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<tr>
<td>MFI</td>
<td>3 (2.54-3)</td>
<td>2.7 (1.8-3)</td>
<td>2.5 (1.9-3.0)*</td>
<td>2.8 (2-3)*</td>
<td>2.6 (2-3)</td>
<td></td>
</tr>
<tr>
<td><strong>Gastric</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TVD</td>
<td>20.6 (14.5-28.7)</td>
<td>X</td>
<td>13.5 (9-21)*</td>
<td>16.3 (13-24)*</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>PVD</td>
<td>20.6 (12.9-28.5)</td>
<td>X</td>
<td>7.1 (0-18)*</td>
<td>15.5 (7-21)*</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>PPV</td>
<td>100 (86.87-100)</td>
<td>X</td>
<td>51.4 (3-96)*</td>
<td>96.6 (37-100) †</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MFI</td>
<td>3.0 (2.75-3.0)</td>
<td>X</td>
<td>1.4 (1-3)*</td>
<td>3 (1-3) †</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 Comparison between the median sublingual and gastric microvascular values of the control dogs and GDV dogs as well as between the GDV time points. All values presented as median (range) TVD (vessels/mm)= total vessel density, PVD (vessels/mm) = perfused vessel density, PPV (%) = proportion perfused vessels, MFI = microcirculatory flow index. X = indicates no value available, * = indicates significant difference (p<0.05) compared to control, † = indicates significant difference (p<0.05) between pre-derotation to post-derotation.
Figure 15 Representative still images of microcirculation of a single GDV patient: A = buccal pre-induction mucosa, B = pre-derotation sublingual mucosa, C = post-derotation sublingual mucosa, D = pre-derotation gastric serosa, E = post-derotation gastric serosa, and F = recovery sublingual mucosa.
Figure 16 Box-and-whisker plot of the sublingual microvascular variable total vessel density (TVD) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-induction, pre-derotation, post-derotation, and recovery time points. Statistical significance considered at $p < 0.05$, † = indicates statistical difference of variable compared to the control sublingual mucosal microvasculature.
Figure 17 Box-and-whisker plot of the sublingual microvascular variable perfused vessel density (PVD) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-induction, pre-derotation, post-derotation and recovery time points. There were no statistically significant differences between any of the time points.
Figure 18 Box-and-whisker plot of the sublingual microvascular variable proportion of perfused vessels (PPV) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-induction, pre-derotation, post-derotation, and recovery time points. Statistical significance considered at $p < 0.05$, † = indicates statistical difference of variable compared to the control sublingual mucosal microvasculature.
Figure 19 Box-and-whisker plot of the sublingual microvascular variable microvascular flow index (MFI) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-induction, pre-derotation, post-derotation, and recovery time points. Statistical significance considered at p < 0.05, † = indicates statistical difference of variable compared to the control sublingual mucosal microvasculature.
Figure 20 Box-and-whisker plot of the gastric microvascular variable total vessel density (TVD) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-derotation and post-derotation. Statistical significance considered at p < 0.05; † = indicates statistical difference of variable compared to the control gastric serosal microvasculature, * = indicates statistically different of variable compared to the pre-derotation gastric serosal value.
Figure 21 Box-and-whisker plot of the gastric microvascular variable perfused vessel density (PVD) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-derotation and post-derotation. Statistical significance considered at \( p < 0.05 \), † = indicates statistical difference of variable compared to the control gastric serosal microvasculature.
Figure 22 Box-and-whisker plot of the gastric microvascular variable proportion of perfused vessel (PPV) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-derotation and post-derotation. Statistical significance considered at p < 0.05, † = indicates statistical difference of variable compared to the control gastric serosal microvasculature, * = indicates statistically different of variable compared to the pre-derotation gastric serosal value.
The study revealed that intraoperative assessment of gastric and sublingual microcirculation during a disease state such as GDV is possible using SDM technology. Additionally, evaluation of the microcirculation at several time points may allow for determination of changes in microvascular abnormalities with intervention (such as Figure 23 Box-and-whisker plot of the gastric microvascular variable microvascular flow index (MFI) of the normal dogs and gastric dilatation volvulus (GDV) dogs at pre-derotation and post-derotation. Statistical significance considered at p < 0.05, † = indicates statistical difference of variable compared to the control gastric serosal microvasculature, * = indicates statistically different of variable compared to the pre-derotation gastric serosal value.

3.4 Discussion
This study revealed that the intraoperative assessment of gastric and sublingual microcirculation during a disease state such as GDV is possible using SDM technology. Additionally, evaluation of the microcirculation at several time points may allow for determination of changes in microvascular abnormalities with intervention (such as
gastric derotation). Compared to control dogs, patients with GDV have a significant reduction in gastric microvascular parameters which did improve after derotation. Sublingual TVD was actually increased compared to control with a decrease in PPV and MFI, none of which significantly changed over time. It was also determined that poor correlation may indicate a limited ability for changes in sublingual microcirculation to reflect those occurring in gastric tissues. It was further determined that a poor correlation exists between systemic variables and microvascular variables, suggesting that typical anesthetic monitoring may not be reflective of microcirculatory derangements.

Fitting with previous investigation, our study found that intra-operative evaluation of the gastrointestinal tract is possible in the canine patient. However, as in the previous study, one of the primary difficulties was obtaining quality, uninterrupted videos of 20 seconds duration. As such, the maximum consistent video duration that could be obtained was 5 seconds, which is less than recommended for SDM images. One of the primarily concerns with shortened time of quality video is the risk of over-estimating the quality of the microvascular flow. Given that the short segment of video may capture what is perceived to good flow over 5 seconds, may actually be intermittent or sluggish flow over 20 seconds. This would lead to vessels documented as having good flow when they should be classified as poor quality overall flow and serve to reduced PPV, PVD, and MFI. Our study demonstrated that the ability to obtain SDM videos of the sublingual tissue is strong as all subjects had at least 3 videos of sufficient quality obtained for all patients. However, obtaining videos of pre-derotational gastric
microcirculation was more challenging with only 50% of patients (6/12) having adequate videos. For reasons that are not entirely clear, and despite repeated efforts, it was very difficult to obtain sharp focus of the microvasculature at this time point. This was not experienced when imaging the post-derotation gastric serosa (or when previous controls were performed). It is possible this was a consequence of tissue changes brought about by gastric distention and/or severely impaired blood flow. After derotational, video capture was much more successful, with 92% (11/12) of patients included.

One concern for intra-operative use of SDM is the potential to extend anesthesia/surgery time, especially in an unstable patient. In this study, it was determined that the time to acquire each video was not different between the sublingual and gastric videos (0.75 min/video and 0.74 min/video respectively). The average time to obtain buccal videos was 2.7 minutes, suggesting that pre-operative delay is fairly minimal (though data was only available from 7/12 patients). The attempted time to obtain videos in the other 5 patients was not recorded, however time to assess patient compliance or interference from pigmentation was fairly brief. Extension of anesthesia time from intra-operative video acquisition was approximately 6.5 minutes per patient. Of this time, on average 2.2 minutes was prior to derotation (n=10) and 4.7 minutes was after derotation (n=12). Therefore the delay of gastric derotation was minimal; however even this small delay may be detrimental for some patients, especially those with gastric tissue necrosis or significant cardiovascular instability. Regardless, even in stable patients it has been previously shown that total anesthesia time can have an impact on surgical complications,
including increased risk of infection.\textsuperscript{45} Therefore, patient stability and likely duration of the surgical procedure should serve to dictate whether intra-operative imaging is feasible for the individual patient.

This study supported the use of sublingual microcirculation as an alternative to the previously used buccal mucosa.\textsuperscript{21,41} Owing to limitations associated with a conscious patient and other logistical considerations, buccal microcirculation was used for PrI. As there are challenges with buccal imaging with a patient in dorsal recumbency, sublingual was used for the remaining time points. While these two sites may have different patterns of microcirculation, no significant differences were found among any of the microcirculatory parameters suggesting there are similarities. However, further investigation is warranted. When evaluating the sublingual microcirculation at the numerous time points of this study, no significant differences were found. This could be due to preservation of the sublingual microvascular flow preventing a demonstrated change to the measured microvascular variables. This may be especially true given that, overall, the population of GDV patients in this study were relatively stable, as reflected by only mild increased in lactate and fairly normal blood pressure at the time of presentation and throughout. It is therefore possible that there was not enough systemic compromise to negatively impact the buccal or sublingual microcirculation in this patient population.

Another objective of our study was comparison of the previously established control gastric and sublingual microcirculatory values to similar tissue beds of the GDV
patients. As could be expected, many significant differences were found, including PrI values for TVD, PrS values for TVD, PPV, MFI and all PrG variables. At the post-derotational time points, there was significant difference between sublingual TVD, PPV, MFI and gastric TVD and PVD, and between the recovery TVD values. Specifically within the sublingual microvascular variables, TVD was significantly higher at all time points when compared to the control. However, the PPV and MFI were significantly lower compared to control at PrS. These findings may indicate the response of the tissue beds to a decreased perfusion or flow (PPV and MFI) by increasing the total vessel number (TVD) with no net change in overall perfusion (as reflected by PVD). This could occur via recruitment of additional capillaries in an attempt to continue to deliver oxygen to tissues, especially in the face of increased oxygen demand. Given the nature of GDV, it stands to reason that all gastric microvascular variables at PrG were significantly less than control. After derotation, while TVD and PVD remained decreased compared to control, PPV and MFI were no longer significantly different; suggesting some improvement in gastric perfusion. However, there was still some degree of impairment with failure for all to return to control values. And so while the subjective intraoperative assessment of gastric perfusion suggested normalization after derotation, these findings support the presence of continued microvascular derangement, a notion which is supported by previous publications.29, 44, 46

Our study did not evaluate histopathological changes in GDV patients as only one required a partial gastrectomy. A previous study evaluating colonic torsion in horses,
found that the histopathology on affected tissues had good correlation with microvascular variables.\textsuperscript{43} However, these results of persistent microvascular disease support the questionable nature of subjective tissue assessment. This study suggested the potential benefit of direct microvascular assessment during disease and also demonstration of improvement of the microcirculation (even if not completely normal) as an important indicator of return of blood flow. Additional studies evaluating tissue microcirculation at more time points may be helpful to determine the point at which complete turn towards normal of the microcirculation occurs.

As could be expected, our study did find significant improvement in gastric PPV and MFI between the pre-derotation and post-derotational examinations. This is supportive of previous studies demonstrating an improvement of microcirculation with a concurrent improvement of the clinical state.\textsuperscript{36,47} This likely reflects the removal of multiple factors resulting in the loss of gastric microcirculation. One such factor is the occlusion of the large gastric arteries from both the distention and torsion of the organ. The gastric distention may also lead to an increase pressure within the tissue leading to collapse of the arterioles, capillaries and the venules. Another factor may be the decreased local delivery of oxygen secondary to disruption of blood supply through loss of tissue perfusion arteries, such as the short gastric arteries, from tearing during the dilation and volvulus.

This study demonstrated that the sublingual microcirculation had poor correlation with the gastric microcirculation, with the only significant correlation found with pre-
derotation MFI. This is in contrast to a number of previously studies. One study evaluated sublingual and intestinal microcirculation in patients with abdominal sepsis. This study demonstrated that although there was no correlation between these tissue beds on the first day of monitoring, by the third day both had return to normal flow with significant correlation. Another study evaluating early microcirculatory flow changes in sepsis demonstrated an association between organ failure in the first 24 hours and evidence microcirculatory dysfunction. This study also found that with microcirculatory improvement was associated with a decrease in organ failure. Given this information, it is unclear why in the present study only MFI was found to correlate between the two sites. However, this does suggest that assessment of sublingual MFI may be helpful in monitoring of gastric tissue; as the sublingual MFI decreases, this may be suggestive that the gastric MFI is also decreasing. This finding should be evaluated in additional studies of the gastrointestinal tract.

Given the challenges and limited availability of SDM, it would be helpful if more commonly measures systemic variables were reflective of changes in microvascular perfusion, though previous studies have suggested poor correlation. In the present study, there were a few significant correlations found with some of the respiratory variables, including SpO₂, RR, and EtCO₂. This likely reflects the impact of arterial and tissue concentrations of oxygen and carbon dioxide on vascular tone. There is evidence to suggest that an increase in oxygen tension leads to vasodilation and therefore an increase in the PVD of that tissue, which may account for the positive
correlation between the PrG PVD and pulse oximetry values and the positive correlation of the PrS PPV and ScvO₂. This correlation may be due to gastric microvascular shunting at the tissues or at the capillaries prior to the gastric tissue, given the decreased flow to the distended tissues altering the normal capillary autoregulation. The positive correlation between PrS MFI and EtCO₂ and the negative correlation of the Rec respiratory rate and MFI may reflect the natural response of the microcirculation to vasodilate in response to increased arterial concentration of carbon dioxide. Finally, when considering markers of poor tissue oxygen delivery, such as lactate, the study found a negative correlation of the PtG MFI and lactate. This may support the notion that improved perfusion to the stomach as result of derotation would lead to a decrease in lactate. Interestingly however, the study results also demonstrated a positive correlation between PtS TVD and PVD and lactate, which is the opposite of expected. One possible explanation is that improved perfusion after an ischemic event (such as GDV) could result in increased liberation of lactate from tissue, and thereby a positive correlation. Alternately, as there was no significant change in sublingual parameters over time, it may suggest that cephalic circulation was maintained independent of changes in gastric perfusion and the positive correlation was found by chance and one does not truly exist. This is supported by the fact that multiple studies in human patients have demonstrated a lack of correlation between lactate and microvascular parameters. Overall, assessment of macrovascular and microvascular correlation yielded some interesting and conflicting results. Further studies would need to be performed to determine the persistence of these findings.
There are some limitations for this study. Given that these patients were undergoing surgery, some impact of the anesthetic protocols used is unavoidable. For example, inhalant anesthetics and propofol may cause vasodilation,\cite{49,50,51} and ketamine may cause vasoconstriction.\cite{51} Given that all of these medications were utilized in our study population, the microcirculation may have been affected. Another potential limitation is the cardiovascularly stable nature of our patient population. Given that the overall systemic impact for these patients was fairly mild, the extent of microcirculatory compromise that may be possible in GDV may not have been effectively evaluated. The population examined only included one patient that required gastric resection and no patients died during this study. And while we had intended to exclude any patients that were too unstable for extended anesthesia time, this was not actually necessary for any of the patients enrolled. Therefore, results of this study may not extend to those patients that are more severely affected or that require resection of the gastric tissue. Another limitation is the small sample size. Although we reached the a priori calculated power analysis sample size, additional patients may have allowed the ability to detect more subtle differences, and better assessment for correlation.

The use of the SDM technology has its own inherent limitations. At different time points, it was necessary to image buccal versus sublingual microcirculation for reasons previously discussed. While values obtained appeared to be very similar, without more directed correlation analysis it is difficult to interpret changes over time and with patient positioning. Further it was found that gastric peristalsis and use of cautery interfered
with the ability to obtain videos of the recommended 20 second duration. Microvascular analysis is performed off-line and is labor-intensive. And while subjective assessments can be made at the time of video acquisition, it has been demonstrated that these can have poor correlation to actual calculated values.\textsuperscript{52}

3.5 Conclusion

This study demonstrated the feasibility of intraoperative imaging with SDM for patients with GDV, Further, it was determined that there is a significance impact gastric microcirculation with improvement after derotation. Finally we revealed the lack of correlation of the serosal microcirculation and variable correlation of systemic variables to the gastric microcirculation. The use of SDM technology will continue to be of benefit in diagnosis and possibly management of the GDV patient and in other diseases with future studies.

3.6 Footnotes

c = Hydromorphone hydrochloride, West ward, Eatontown, NJ.

d = Methadone, Mylan Laboratories, Canonsburg, PA.

e = PropoFlo, Abbott Laboratories, North Chicago, IL.

f = Ketamine hydrochloride, Pfizer, Inc., New York, NY.

g = Fluriso, MWI, Boise, ID.

h = Cardell® MAX-12 DUO HD Multiparameter Monitor, Midmark Corp, Versailles, OH.
i = Lactated Ringer’s Solution, Baxter Healthcare, Deerfield, Ill.

j = Model 811-B Doppler Ultrasound, Parks Medical Electronics, Inc., Aloha, OR.

k = Lactate Plus, Nova Biomedical Corporation, Waltham, MA

l = Microscan, MicroVision Medical, Amsterdam, The Netherlands.

m = Ag-Tek® MaxiSleeve®, Neogen® Corporation, Lexington, KY.

n = MediChoice® Endoscopic Anti-Fog Solution, Owens & Minor, Richmond, VA.


r = IBM® SPSS Statistics Version 21

s = Midazolam injectable, Hospira Inj, Lake Forest, IL

t = SevoFlo, Abbott Laboratories, North Chicago, IL

u = Fentanyl citrate Injectable, Hospira Inj, Lake Forest, IL

v = Arrow International Inc, Reading, PA

w = Vetstarch®, Abbott Laboratories, North Chicago, IL

x = Mindray Datascope Passport® V, Mindray DS USA Inc, Mahwah, NJ

y = NOVA CCX, Nova® Biomedical Corporation, Waltham, MA

z = pHox Ultra, Nova® Biomedical Corporation, Waltham, MA

aa = Tidal Wave Sp, NOVAmetrix, DRE Medical, Inc, Louisville, KY
3.7 References

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Conclusion
Within the body, the microcirculation, consisting of the arterioles, capillaries and venules measuring <200 μm, is vital for delivery nutrients, such as oxygen, and removing waste products. Given the key role of the microcirculation in tissue health, there has been growing interest in evaluating its role in disease and response to therapy both in research and in clinical studies. Advancements have been made to allow direct evaluation of the vessels, including the technology of sidestream dark field microscopy (SDM). With this technology and its ability to have both qualitative and quantitative assessment of the microcirculation, there are many potential applications both in human and veterinary medicine.

Through the course of these investigations, it was determined that intra-operative use of SDM technology in dogs is feasible for evaluation of the sublingual mucosa and the gastric serosa microcirculation and successful techniques for each were established. Through the use of a control population of healthy dogs undergoing elective gastropexy, reference values for microcirculatory parameters were established. For the sublingual mucosa, these included (median (range)): TVD 28.0 vessel/mm (21.9-31.3), PVD 26.5 vessel/mm (20.7-31.2), PPV 96.8 % (87.5-100), and MFI 3 (2.54-3). For gastric serosa these included: TVD 20.6 vessel/mm (14.5-28.7), PVD 20.6 vessel/mm (12.9-28.5), PPV 100 % (86.87-100), and MFI 3 (2.75-3). When comparing microvascular values between
the tissue beds, there were statistically significant differences between the TVD and PVD values, whereas there were no differences with PPV or MFI. This suggests that a greater number of blood vessels are present in sublingual mucosa, but both tissue beds are kept well perfused. It was also found that there is no significant correlation between the two tissues, suggesting that changes in one are not necessarily reflected in the other. When comparing microvascular parameters to systemic variables, respiratory values (respiratory rate, end-tidal carbon dioxide and pulse oximetry) were found to have a significant negative correlation, but no association was found with hemodynamic assessments. While these values are in normal dogs, this study has set the stage for the information to be used as a basis of comparison for microvascular investigation into other surgical and gastrointestinal diseases.

A second portion of this project involved the evaluation of sublingual mucosa and gastric serosa in dogs with naturally occurring gastric dilatation volvulus (GDV). In order to determine the impact of disease and therapy, patients were assessed at various time points during the disease process. The time points were admission, pre-induction, pre-derotation, post-derotation, and recovery. Comparing the sublingual microvascular variables across time points revealed no significant differences, despite anesthesia, surgical intervention, gastric derotation, and subsequent recovery. However, when comparing the microvascular variables for gastric tissue between pre-derotation and post-derotational, there was significant improvement of the PPV and MFI, but the TVD and PVD variables were not statistically different. This suggests that the total number of
vessels did not increase after de-rotation, but their effective perfusion did. Microvascular parameters points were compared to the previously established control values for all time points and locations. It was determined that GDV patients had significantly higher sublingual TVD but lower PPV when compared to control dogs. This likely indicates recruitment of new vessels in the face of decreased vessel perfusion to maintain PVD. Further, GDV patients had significantly lower gastric microvascular values prior to derotation. While TVD and PVD improved after derotation, they were still significantly less than control values. The values for PPV and MFI, on the other hand, were no longer significantly different from control after derotation. When comparing the sublingual mucosal and gastric serosal microvascular parameters a significant correlation was found for MFI, but not any of the other values. Correlations between microvascular and systemic variables, including heart rate, end-tidal carbon dioxide, pulse oximetry, and respiratory rate, along with variables evaluating tissue oxygenation status, central venous oxygen saturation and lactate, were found to significant at various time points.

These studies allowed for many key revelations regarding use of SDM in veterinary patients. One is the successful evaluation of the sublingual mucosa and an intra-operative evaluation of the gastric mucosa, while maintaining sterility. It was also determined that there was a lack of correlation between the sublingual and gastric microcirculatory variables for both study populations. Further, there was a lack of clinically relevant correlation between systemic variables and microvascular parameters in both the normal and GDV dogs. Finally, we established that the microcirculation is
deranged within GDV dogs at all time points. While there was improvement after derotation, some values were still significantly worse than the control population, suggesting persistent disruption of microvascular perfusion.

With these reported results, we hope to shed light on the importance of microvascular perfusion and the benefits of direct evaluation of the microcirculation. This study has only evaluated one particular disease process, gastric dilatation volvulus, but many more diseases exist that could be associated with microcirculatory impairment. For future directions, there would likely be benefit from continued examination of microvascular function for these diseases and the potential impact of diagnosis, treatment, and future prevention.
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