The effects of sildenafil on portal vein velocity, cross-sectional area, and congestion index in the dog

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The effects of sildenafil on portal vein velocity, cross-sectional area, and congestion index in the dog

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The main use of sildenafil in human medicine is to treat erectile dysfunction. In veterinary medicine, sildenafil is most frequently used to treat pulmonary hypertension. The effects of sildenafil on the portal vasculature in the dog have not been previously evaluated. The purpose of this study was to evaluate the effects sildenafil has on the portal vasculature. The cross-sectional area of the aorta, cross-sectional area of the portal vein, and portal vein velocity were acquired in thirteen dogs prior to administration, 45 minutes, 90 minutes, and 120 minutes after the oral administration of sildenafil for the treatment of pulmonary hypertension. No statistically significant difference was detected between all measured values at all time points. Although this study had a small sample size, sildenafil does not have a significant effect on the size of the portal vasculature. Further studies with a larger sample size will be required to further evaluate the effects.
TABLE OF CONTENTS

LIST OF TABLES ......................................................................................................................... iii
LIST OF FIGURES ......................................................................................................................... iv

CHAPTER

I.  INTRODUCTION ......................................................................................................................... 1

  Hepatoportal vasculature anatomy ............................................................................................... 1
  Pertinent hepatoporal disease ...................................................................................................... 9
  Portal vein hypertension .............................................................................................................. 9
  Sildenafil .................................................................................................................................. 13
  Monitoring and evaluation of the portal system ......................................................................... 17

II.  THE EFFECTS OF SILDENAFIL ON PORTAL VEIN VELOCITY, CROSS-SECTIONAL AREA, AND CONGESTION INDEX IN THE DOG ........................................... 23

  Study objectives ....................................................................................................................... 23
  Hypothesis .............................................................................................................................. 23
  Materials and methods ............................................................................................................ 23
    Study population .................................................................................................................. 23
    Imaging and measurements ................................................................................................. 24
    Statistical methods .............................................................................................................. 27
  Study results ........................................................................................................................... 27
    Study population results ...................................................................................................... 27
    Imaging results ..................................................................................................................... 28
  Discussion ............................................................................................................................... 29
    Conclusions ......................................................................................................................... 29
    Limitations .......................................................................................................................... 32
    Future studies ...................................................................................................................... 33
    Synopsis ................................................................................................................................ 33

REFERENCES ............................................................................................................................ 34
LIST OF TABLES

Table 2.1  Study Population ........................................................................................................28
Table 2.2  Average portal vein area, aorta, and portal vein area to aorta ratio ..................28
Table 2.3  Average portal vein velocity and congestion index over time .......................29
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Canine abdominal arterial system. Image adopted from Evans.²</td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>Portal venous system of the dog. Image adopted from Evans.²</td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>Branching of the portal vein within the liver. Modified from The Human Body.⁴</td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td>Cellular level of the liver. Image adopted from Levy.⁶</td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>Chemical composition of sildenafil citrate</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>Angle of insonation</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>Right lateral intercostal image of the porta hepatis</td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>Portal vein velocity</td>
<td></td>
</tr>
</tbody>
</table>
Hepatoportal vasculature anatomy

The blood supply to the liver is unique as the liver receives blood from two different sources, the hepatic artery and the portal vein.\(^1\) As oxygen rich blood leaves the left ventricle, it enters into the aorta.\(^2\) The abdominal portion of the aorta has three unpaired arteries (celiac, cranial mesenteric, and caudal mesenteric).\(^2\) The first unpaired artery, the celiac artery, divides into the hepatic artery, left gastric artery, and the splenic artery.\(^2\) The hepatic artery further divides into hepatic branches which perfuse the hepatic parenchyma, and a cystic artery which courses towards the gallbladder.\(^2\) (Figure 1.1)
Figure 1.1  Canine abdominal arterial system. Image adopted from Evans.²

The canine abdominal aorta. The aorta originates from the left ventricle then traverses the diaphragm (not imaged). The first unpaired branch from the aorta is the celiac artery (blue oval). The celiac artery branches into the left gastric, hepatic and splenic arteries. The hepatic artery (blue arrow) has several branches; right gastric, gastroduodenal, and multiple hepatic branches.
The portal vein is a venous system that returns blood from the stomach, small intestines, colon, pancreas, and spleen and brings absorbed nutrients from the gastrointestinal tract. Nutrient rich blood from the small intestines converges from the jejunal veins into the cranial mesenteric vein. The cranial mesenteric vein continues towards the liver, receiving blood from the caudal pancreaticoduodenal and caudal mesenteric veins. Just before entering the liver, the splenic vein and the gastroduodenal vein join the portal vein. The liver receives approximately 75-80% of its blood supply from the portal vein and the remainder from the hepatic artery. In terms of oxygen being carried in the blood, the hepatic artery supplies approximately 30-40% of the total oxygen to the liver. (Figure 1.2)
Figure 1.2  Portal venous system of the dog. Image adopted from Evans.²

The portal vascular system of the dog. The jejunal veins anastomose to create the cranial mesenteric vein (red arrow). The caudal mesenteric, caudal pancreaticoduodenal, and ileocolic veins provide blood to the cranial mesenteric veins which combine to form the portal vein (red oval). As the portal vein courses cranially, the splenic vein and gastroduodenal veins provide blood to the portal vein prior to the portal vein entering the liver.

As the portal vein enters the liver, it divides into a short right branch and a long left branch.² (Figure 1.3) The right branch provides blood to the right lateral and caudate process of

4
the caudate lobe. The left branch provides blood supply to the right lateral, quadrate, left medial, and left lateral lobe. The left branch is subdivided into a left lateral and left medial portion.

Figure 1.3 Branching of the portal vein within the liver. Modified from The Human Body.

The portal vein divides into a left and right branch.

As blood from the terminal branches of the hepatic arteries and portal vein extend into the liver, they form hepatic triads which then flows from the triad towards a central vein. Portal triads are made of branches of the portal vein, hepatic arteries, and biliary ducts. Before the blood reaches the central vein, the blood from the portal veins and hepatic arteries mix in the hepatic sinusoids. (Figure 1.3) The liver in turn, has cells called the Kupffer cells which are hepatic macrophages, that live in the sinusoidal lining that are crucial to remove foreign substances, bacteria, and dying cells from the blood in circulation. These cells also can act as antigen presenting cells, to help bolster the immune response. After the blood in the liver reaches the central vein, it anastomoses with other central veins from other regions of the liver to
form the hepatic veins. The hepatic veins then anastomose to create the caudal vena cava which returns blood to the right atrium.⁵

![Figure 1.4](image-url)  
**Figure 1.4**  Cellular level of the liver. Image adopted from Levy.⁶

Branches of the portal vein and the hepatic arteries join to make a common vessel that leads to the central vein. The central vein collects from each portal triad and joins to form the hepatic veins.

A study performed by Luatt et. al. evaluated the hepatic buffer system using cats as a model.⁷ They placed electromagnetic flow probes in the hepatic artery and superior mesenteric artery. The blood the portal vein receives is ultimately from the cranial mesenteric artery, thus in this study, it was used in place of directly catheterizing the portal vein.⁷ A clamp was placed on the vessels to restrict the flow. The authors found when portal vein blood flow was restricted,
there was a linear increase in blood flow from the hepatic artery into the hepatic parenchyma. Adenosine, a strong vasodilator, was infused into the portal vein. No additional changes in the pressure or blood flow of the hepatic artery were produced. Additionally, through blood flow redirection, increased blood was routed through the cranial mesenteric artery. In response, a linear correlation of the hepatic artery constricting was identified. Further arterial constriction was not identified after the infusion of norepinephrine, a potent vasoconstrictor. With this study, the authors were able to prove the direct effects of the portal vein blood flow and pressure has on the hepatic artery blood flow and pressure. Furthermore, the authors evaluated prior studies and found similar findings that provided a linear and nonlinear correlation, but upon critical evaluation of the statistics of the nonlinear models, concluded the buffer system has a linear relationship. Some drugs such as glucagon, isoproterenol, and adenosine cause dilation of the cranial mesenteric artery, but at specific doses decrease the blood flow within the hepatic artery. But in further studies that evaluated this finding, they concluded the buffer system was the cause for the decreased blood flow within the hepatic artery, and when removed via mechanical or chemical blockades, these drugs showed normal dose related dilation to the hepatic artery. This study and its predecessors conclusively established the buffer system and the effects it has on the blood flow to the liver.

On a molecular level, there are several different agents that affect the vascular tone. Nitric oxide (NO) is a chemical signal for cells that is found throughout the body and has different responses depending on the cell. The arterial vascular endothelium produces NO which is then released into the interstitium where it interacts with the vascular smooth muscles. Within the smooth muscle, NO causes a reaction that activates and increases the amount of cGMP. Cyclic guanosine monophosphate causes dephosphoralation of myosin which cause myosin to
disassociate from actin. This disassociation leads to muscle relaxation. After completion of this cycle, cGMP is broken down by cGMP phosphodiesterase. Because of this reaction, NO is a main contributor to vasodilation.

Endotoxins such as guanosine triphosphate are also able to cause arterial dilation. This endotoxin produces tetrahydrobiopterin which enhances endothelial nitric oxide synthases, which follows along the same tract as exogenous NO. Additionally, tissue necrosis factor alpha is produced by mononuclear cells as a result of bacterial endotoxins. Tissue necrosis factor alpha has been proven to cause a release of NO, the mechanism of action is unknown, but it may stimulate tetrahydrobiopterin.

Similar to NO, increased carbon monoxide (CO) increases the cGMP with in the vascular smooth muscles. In the brain, CO and heme oxygenase are likely the cause of vasodilation, not NO.

Prostacyclin is a product of cyclooxygenase from the endothelium. This enzyme causes relaxation of smooth muscle and has been identified to be in elevated levels in rabbits with portal vein hypertension and patients with cirrhosis.

Arterial endothelium can become hyperpolarized with endothelium-derived hyperpolarizing factor which can cause vasodilation. Additionally, endocannabinoids have receptors on the mesenteric vasculature and can cause hypotension. This arterial dilation can then cause portal hypertension through the “forward flow” theory described later.

Adrenomedullin is a vasodilator that has been identified in patients with hepatic cirrhosis and elevated plasma nitrites. This protein increases cGMP production.
The aorta and mesenteric arteries also have dilatory effects due to hydrogen sulfide.\textsuperscript{8} Hydrogen sulfide opens K<sub>ATP</sub> channels which produces a transient decrease in blood pressure.\textsuperscript{8}

In addition to the increasing the production of cGMP, another method to produce vasodilation is by blocking its degradation. One major pharmaceutical of interest that utilizes this pathway is sildenafil which is discussed below.

**Pertinent hepatoportal disease**

**Portal vein hypertension**

Portal vein hypertension is defined as a hepatic venous pressure gradient between the portal vein and the caudal vena cava exceeding 5 mmHg.\textsuperscript{9} When this pressure gradient becomes greater than 10 mm Hg, varices or portosystemic collateral circulation can begin to form.\textsuperscript{9} When evaluated using ultrasound, a velocity of less than 10 cm/s is deemed to be portal vein hypertension.\textsuperscript{9}

There are several broad categorical causes of portal vein hypertension, including prehepatic, intrahepatic, and posthepatic.

Portal vein hypertension that is caused by prehepatic etiologies include abnormalities anywhere from the capillary beds that lead into the portal vein and through the portal vein to the liver. Several examples of prehepatic portal vein hypertension include congenital absence of the portal vein, intraluminal obstruction, and extraluminal obstructions.\textsuperscript{1,10} Congenital absence of the portal vein (atresia) is due to failure of the vitelline veins to anastamose with the hepatic sinusoids or due to involution of the periduodenal vitelline veins.\textsuperscript{10} In this situation, the portal vein directly communicates with the caudal vena cava and shunts blood around the liver.\textsuperscript{10} In cases of portal vein aplasia, or lack of portal vein formation, the portal vein does not continue into the portal triads in the liver which can be confirmed on histology.\textsuperscript{10} Intraluminal and
Extraluminal portal vein obstructions can cause either partial or complete blockage of blood as it leaves the gastrointestinal tract and enters the liver. Intraluminal obstructions can be caused by thrombus formation or neoplastic infiltration. Extraluminal portal vein obstruction can be caused by neoplasia, granuloma formation, or iatrogenic causes.

Intrahepatic causes of portal vein hypertension include hypoplasia of the portal vein, hepatic nodular hyperplasia, chronic hepatitis, lobar dissecting hepatitis, schistosomiasis, chronic cholangitis, and hepatic arteriovenous fistula. Portal vein hypoplasia is when the portal vein branches into the liver, but the hepatic branches of the portal vein at the triads are underdeveloped. This can be divided as a primary cause or secondary. Where primary portal vein hypoplasia is when the portal vein does not develop properly, secondary portal vein hypoplasia is when the portal vein is underperfused, usually due to a portosystemic shunt. Ailments that increase resistance within the hepatic parenchyma such as nodular hyperplasia, chronic hepatitis, lobar dissecting hepatitis, schistosomiasis, and chronic cholangitis can all lead to portal vein hypertension. These etiologies can cause increased sinusoidal pressure in the hepatic triads which requires elevated portal and arterial pressure to produce forward blood flow.

Unlike the other causes of intrahepatic causes, hepatic arteriovenous fistula occurs when the portal vein anastomoses with the arterial system. This can be referred to as high velocity shunting as the blood in the arterial system is of higher velocity and pressure than the portal system which can lead to blood entering the portal system directly from the arterial system. Fortunately, this condition is rare in dogs and cats and may not show clinical signs until hepatic encephalopathy or portal vein hypertension arise.

Obesity has also been associated with increased fat deposition within the liver. Increased fatty infiltration decreases vascular compliance within the liver, thus increasing the
hepatic resistance. Bellotta et al. performed a study evaluating the effects of obesity on the portal system. Portal blood flow volume was found to be decreased in obese patients which is consistent with human studies. They also found a significant increase of the portal congestion index due to a decrease in mean portal velocity and increase in portal vein area. This study also evaluated the waveform of the hepatic artery and found the normal triphasic waveform of the hepatic artery frequently became biphasic in overweight animals. This finding was likely due to the increased hepatocytes and increased vascular resistance. This finding is similar to what was seen in humans with moderate liver disease, with severe liver disease potentially yielding a monophasic waveform for the hepatic artery.

Posthepatic causes of portal hypertension include right sided heart failure and intraluminal and extraluminal obstructions of the caudal vena cava. These causes prevent the forward flow of blood from the liver to the right side of the heart. The result is blood retained within the liver and increase hepatic resistance, which in turn leads to portal vein hypertension.

A theory for early or mild portal vein hypertension is the “backward flow” theory. This theory states due to the increased in hepatic resistance, the splanchnic system is hypodynamic which is supported with the observation of decreased portal vein flow. When the disease advances to moderate or severe portal vein hypertension and collateral circulation is present, decreased blood flow through the portal vein to the liver may be observed, but the patient is truly in a hyperdynamic state. In this state, with increased resistance and increased portal blood flow, the patient may experience further exasperation of portal vein hypertension, which is the “forward flow” theory. Iwakiri, et al further explain that the vasodilation of NO activates sodium retention and plasma expansion by activating the renin-angiotensin-aldosterone system, leading to a hyperdynamic state in the patient. This then further compounds the deleterious
effects of diseases such as portal vein hypertension. Hepatopulmonary syndrome or portal-pulmonary syndrome results from hyperdynamic state and this increases shear pressure to the pulmonary system. 

Once portal vein hypertension is established, complications such as ascites or abdominal fluid accumulation, portosystemic shunts, decreased velocity of portal blood flow, and dilation of the portal vein may develop. Increase in the hydrostatic pressure within the portal vein can lead to leakage of plasma from the portal vein into the peritoneal space of the patient. This results in peritoneal effusion or ascites. Additionally, as the increased pressure within the portal vein continues to elevate, other organs such as the pancreas can become edematous.

Portal vein hypertension is a leading cause for acquired portosystemic shunts. These acquired shunts are variable in location and appearance. In the altered hemodynamic state, angiogenetic factors can lead to the formation of new vessels in an attempt to divert blood from the elevated pressure system and return it to the systemic circulation. Additionally, pre-existing vascular pathways can become patent. There are three described embryologic connections, the left colic-pudendal vein, left gastric-cardiac esophageal branches, and phrenic-portal vein. Another phenomenon that can occur is cavernous transformation of the portal vein. In this situation, the portal vein forms collateral circulation around a portal vein obstruction such as a thrombus.

A study was performed in humans by Villanueva et al. which looked at 294 people with compensated hepatic cirrhosis. This study compared patients with early compensated cirrhosis and those that were free of clinical signs. Those with the clinical signs tended to have higher cardiac output, cardiac index, and lower systemic vascular resistance. Given this difference in hemodynamic state, beta blockers were found be markedly effective, approximately twice the
reduction, on the hepatic venous pressure gradient in patients with clinical signs than those without clinical signs. Early in portal vein hypertension, the elevated pressures are due to the hepatic resistance, restricting the forward flow of blood. As the hypertension progresses, the hyperdynamic state beings to drive the hypertension through increased venous inflow. The authors summarize by stating the early phase of portal vein hypertension is driven by sinusoidal remodeling, endothelial dysfunction, nodular formation, fibrillar extracellular matrix formation, and vascular occlusion which may be better treated by other pharmaceuticals. Unfortunately, there is a dearth of knowledge in medical treatment of portal vein hypertension in the subclinical patients in veterinary medicine.

Typically, treatment of portal vein hypertension is variable depending on the underlying cause. Direct management of the inciting etiology is the primary goal, but symptomatic treatment may be of benefit. Nonselective beta blockers are one of the primary pharmacologic agents utilized for treatment of variceal hemorrhage in humans. Beta blockers cause mesenteric arterial vasoconstriction, decreasing the inflow of blood to the portal system and reducing the portal pressure. This method is the main long-term treatment of choice for portal vein hypertension in humans. Another speculative treatment is sildenafil.

**Sildenafil**

There are 9 phosphodiesterase families. Phosphodieserases are a group of enzymes that have a strong effect by amplifying cyclic nucleotides. These phosphodiesterase inhibitors predominantly affect cyclic adenosine monophosphate (cAMP) and cGMP. The exact effect will vary depending on the species, target organ, and the phosphodiesterase family. The different families have varying affinity for each enzyme thus also having different outcomes when administered. Phosphodiesterase-1 (PDE-1) for example, has a higher affinity for cAMP.
PDE-1 is widely distributed in the brain and heart, whereas phosphodiesterase-6 is involved with light transduction and vision. Although the exact effects of these families vary, they all involve amplifying cyclic nucleotides. Another well known family is phosphodiesterase-5 (PDE-5).

Sildenafil is a selective concentration-dependent, phosphodiesterase-5 (PDE-5) inhibitor that allows for retention of cyclic guanosine monophosphate (cGMP) and amplifies the effects of NO in the target tissues, leading to vasodilation. Sildenafil has a half-life of 4 hours and peak efficacy in 0.5-2 hours in humans and dogs. Phosphodiesterase-5 is present with arterial smooth muscle, but in people, PDE-5 messenger riboneuclai acid (mRNA) was found through chromosome mapping in aortic smooth muscle, myocardium, placenta, skeletal muscle, pancreas, and, to a lesser extent, the brain, liver, and lung. Some of these phosphodiesterase families have the ability to cross-react with other families which can lead to a compounded effect. Sildenafil can weakly inhibit PDE-1 and phosphodiesterase-3 which is also in smooth muscles, platelets, cardiac tissues, liver, fat, and corpora cavernosa.

![Chemical composition of sildenafil citrate](image)

**Figure 1.5**  Chemical composition of sildenafil citrate
Chemical composition of sildenafil citrate. Image adopted from Elnaggar.
Because of these effects, sildenafil is commonly used as a treatment for erectile dysfunction and pulmonary hypertension in humans. Additionally, sildenafil can be utilized in the treatment of idiopathic megaesophagus and ischemic reperfusion injury.

A study performed by Quintavalla randomized 21 puppies that had clinical and radiographic evidence of megaesophagus were treated with sildenafil or a placebo. Food intake was monitored and follow up radiographs to monitor the esophagus were performed. This study found dogs treated with sildenafil had decreased clinical signs of megaesophagus, decreased regurgitation, decreased esophageal diameter on radiographs, and increased body weight. The authors hypothesized that esophageal neurons release NO which increases the cGMP and causes relaxation of the lower esophageal sphincter. This allows for food to pass through the esophagus and into the stomach with ease. Sildenafil allows for further relaxation of the lower esophageal sphincter by decreasing the break down of cGMP. Similar studies were performed in humans and cats which showed the administration of sildenafil caused decreased peristalsis of the esophagus. In humans and cats, the esophagus is composed of smooth muscle, while in dogs it is made of striated muscle. Since sildenafil has an effect on smooth muscle, cats and humans may experience decreased peristalsis contractions of the esophagus while the striated muscle of the dog is not affected.

Gori and associates investigated the effects of sildenafil with ischemic reperfusion injury. Several preliminary studies in rabbits showed sildenafil had a protective capability by limiting the endothelial dysfunction in the ischemic state thus limiting tissue necrosis. Additionally, prior studies had predominantly investigated in cardiomyocytes. This information was extrapolated to humans and 10 people induced radial ischemic insult. Half the study population received sildenafil and the other half a placebo. This process was repeated one
week later with the participants receiving the opposite treatment the second time. The authors evaluated endothelium relaxation and found pretreatment with sildenafil prevented ischemic reperfusion.\textsuperscript{18}

An additional clinical entity, portopulmonary hypertension, is defined as pulmonary arterial hypertension in patients with concurrent portal hypertension. There have been mixed results when using sildenafil to treat people with portopulmonary hypertension prior to liver transplants, with reports demonstrating both improved\textsuperscript{19,20} and aggravated\textsuperscript{21} hypertension.

In a case report by Makisalo et al., a 41-year-old woman presented with an encephalopathy episode.\textsuperscript{20} She was diagnosed with biliary cirrhosis five years prior. She was clinically at end-stage liver cirrhosis with esophageal varices. She had experienced fatigue and muscle convulsions. She was given sildenafil orally 50mg, three times a day. After one week, she was feeling better and after three weeks, her clinical signs had resolved. She went on to receive a liver transplant and was able to return to normal daily activities. The authors hypothesized the cause of the patient’s clinical signs were due to poor oxygenation and decreased hepatic blood flow.\textsuperscript{20}

In a case report by Wang et al., a 37-year-old man with a three-year history of liver cirrhosis presented with esophageal variceal bleeding.\textsuperscript{21} He was not taking beta blockers. He had clinical symptoms of portal vein hypertension. Additionally, the patient was diagnosed with moderate pulmonary hypertension. He was then given 50mg of sildenafil orally. Hemodynamic values were measured before and an hour after oral administration of sildenafil. A mild elevation of wedge hepatic vein pressure (WHVP), hepatic venous pressure gradient (HVPG), hepatic blood flow, cardiac output, heart rate, and decreased systemic vascular resistance and decreased mean pulmonary arterial pressure were observed.\textsuperscript{21} The authors hypothesized nitric oxide (NO)
overproduction plays a role in the hyperdynamic state and as phosphodiesterase 5 is present on the mesenteric arteries, it decreases the vascular tone increasing the blood flow to the portal system further elevating the portal pressures.\textsuperscript{21}

Although portopulmonary hypertension has not been recognized in veterinary patients, some studies have attempted to investigate the effects of sildenafil. One study performed on rats with medically induced portal hypertension showed sildenafil caused an increase in portal vein pressure and mesenteric blood flow.\textsuperscript{22} Despite this study, there remains a dearth of knowledge that sildenafil may have on the canine portal system, particularly in the early or subclinical phase of the disease.

**Monitoring and evaluation of the portal system**

Intraluminal and extraluminal obstructions both prehepatic and posthepatic cause a decrease in the diameter of the vessel which increases the resistance. If the portal vein blood flow is unchanged, this will result in increased portal vein pressure. This is explained by Ohm’s law.\textsuperscript{1}

\[ P(\text{pressure}) = Q(\text{blood flow}) \times R(\text{resistance}) \]  \hspace{1cm} (1.1)

Additionally, this law states that if there is increased resistance through vascular obstructions or more commonly, increased hepatic resistance, the pressure will increase.

When monitoring the hepatoportal system, one of the key features that can be evaluated is the hepatic venous pressure gradient (WVPG). The hepatic venous pressure gradient is the gold standard when evaluating the hepatic sinusoidal pressure and represents the pressure difference between the portal vein and the abdominal caudal vena cava.\textsuperscript{9,23} This measurement is
done by finding the difference between the free hepatic vein pressure (FHVP) and the wedge hepatic vein pressure (WHVP). The free hepatic vein pressure is the measurement of the pressure just beyond the liver parenchyma typically within the right hepatic vein and the WHVP is the hepatic sinusoidal pressure which is typically a close approximation of the portal vein pressure.

\[ HVG = WHVP - FHVP \] (1.2)

When the HVPG is greater than 5 mmHg, portal vein hypertension is present as discussed above. This measurement is performed by catheterizing the antecubital, femoral, or right jugular veins. To acquire this measurement, the tip of the catheter is thread through the vasculature to the right hepatic vein. The FHVP is measured at that site. Then the WHPV is measured by inflating the balloon catheter and measuring the pressure. Unfortunately, this method of monitoring the portal pressure is invasive and not practical in most clinical situations. Issues also arise as this pressure gradient may not be an accurate representation for post hepatic portal hypertension. A more accessible and practical way to measure the portal pressure is indirectly through ultrasound evaluation.

Duplex Doppler sonography has been shown to be an excellent noninvasive alternative for evaluation of the portal vasculature. This utilized color to depict the flow of blood on top of the two-dimensional grey scale image. Despite the portal system being well-described in the literature with conventional B-mode and Doppler ultrasonography, direct or indirect evaluation of portal pressures is often excluded during routine abdominal scans. Due to invasiveness and difficulty of portal pressure measurements, secondary signs of portal hypertension are typically identified (including ascites, portosystemic shunts, decreased velocity of portal blood flow,
dilation of the portal vein, and dilation of the left gonadal vein) rather than direct or indirect measurements.\textsuperscript{27}

Further evaluation of the portal vein velocity can be performed with spectral Doppler. Spectral Doppler ultrasonography utilizes the reflection of ultrasound wave on flowing blood to determine the direction and speed of the moving cells.\textsuperscript{3} In general, the ultrasound transducer produces a sound wave and a portion of this wave is reflected back to the transducer after it reaches a tissue interface.\textsuperscript{3} The ultrasound machine mathematically calculates the distance and amount of the wave that returns to project an image on the screen.\textsuperscript{3} With spectral Doppler, the velocity of the moving cells is plotted over time.\textsuperscript{3} This allows for a waveform to be produce and evaluated by the sonographer.\textsuperscript{3} When performing spectral Doppler, the optimum angle to evaluate the vasculature is parallel to its long axis.\textsuperscript{3} This is not typically feasible, but the angle of evaluation of the structure, or the insonation angle should be below 60°. At approximately 60°, there is a 12% error to the measured velocities, while at 30, this error is reduced to 5.4%.\textsuperscript{28} (Figure 1.5)
Figure 1.6 Angle of insonation

The maroon tube depicts a blood vessel and the grey orb depicts the ultrasound transducer. Sound waves leave the transducer and some are reflected when the interact with the blood vessel wall and the blood cells inside the wall. The black curve is the insonation angle which is the angle of the transducer to the object being imaged. This angle is ideally less than 60° to reduce the amount of error in the calculated velocity.

A waveform is typically evaluated while acquiring the velocity. The portal vein has a monophasic flow which is continuous and flat.\textsuperscript{11,29} There are mild variations in the reported mean velocity in normal dogs; 18±8 cm/s,\textsuperscript{29} 14.7±2.5 cm/s through 18±7.6cm,\textsuperscript{30} 18.1±7.6,\textsuperscript{31} and 15.8 cm/s.\textsuperscript{32}

Another method to evaluate the portal vein is to measure the difference in size of the portal vein vs the aorta or the caudal vena cava. By evaluating the portal vein as a ratio instead of a direct measurement helps to obtain a normal ratio that can be used in dogs off all different sizes rather than having multiple measurements for each size of dog. For this, a normal portal vein to aorta diameter ratio can be obtained. Normal portal vein to aorta diameter ratio is 0.71 – 1.25.\textsuperscript{29}

As with duplex Doppler, this in itself should be interpreted with other findings as the portal vein
The congestion index is another method that is used to evaluate the portal vein. This is a more accurate measurement for portal hypertension as it incorporates the portal vein velocity and the portal vein area.\textsuperscript{25} Measurements of the cross-sectional area of the portal vein and the velocity of the portal vein are required to calculate the congestion index.\textsuperscript{30}

\[ CI (cms) = \frac{(area)^2}{V_{mean} (cm/s)} \]  \hspace{1cm} (1.3) 

It has been shown to be elevated in patients with hepatic disease.\textsuperscript{30} This is deemed to be a better measurement for diagnosing portal vein hypertension than duplex Doppler evaluation alone.\textsuperscript{25} In veterinary medicine, the congestion index has been shown to be variable among patients of different body sizes.\textsuperscript{30} Smaller dogs (<10kg) had a higher congestion index and larger dogs (>20kg) had a lower congestion index.\textsuperscript{30} These findings were due to the area of the portal vein becoming smaller with the smaller patients, but the velocity remained similar amongst all patients.\textsuperscript{30} A prior study performed by Nyland and Fisher found the congestion index to be 0.40±0.15 in healthy Beagles.\textsuperscript{31} The study by Sartor found the congestion index to be 0.022±0.01 (>10kg), 0.039±0.009 (10-20kg), and 0.043±0.009 (<20kg).\textsuperscript{30}

These less invasive methods of evaluating the portal vasculature are the mainstay of monitoring and evaluating the portal system. Additionally, these measurements can be used to monitor the treatment effects of patients with hepatic cirrhosis.\textsuperscript{25} Given the accuracy of these
indirect measurements; they provide an excellent way to study the effects novel drugs may have on the portal vasculature.
CHAPTER II
THE EFFECTS OF SILDENAFIL ON PORTAL VEIN VELOCITY, CROSS-SECTIONAL AREA, AND CONGESTION INDEX IN THE DOG

Study objectives
The aim of this study was to describe the change in cross-sectional area of the portal vein relative to the aorta and the congestion index after the administration of sildenafil. An additional objective was to determine if a significant change in velocity of the portal vein would be identified with ultrasound after the administration of sildenafil.

Hypothesis
The hypothesis was that sildenafil will cause an increase in the portal vein to aorta area ratio due to portal venous dilation. Additionally, sildenafil would cause an increase in portal vein velocity leading to decreased portal vein pressures. With the given increase in portal vein area and increase in velocity, the congestion index of the portal vein would decrease.

Materials and methods
Study population
Client-owned dogs that were going to receive sildenafil as treatment for any presenting condition were recruited. Owner consent was acquired prior to beginning the study. The Institutional Animal Care and Use Committee (IACUC) approved this study prior to data collection, and the study was conducted in accordance with the IACUC protocol (IACUC 18-
If the dog was already being treated with sildenafil, the dog could not have received a dose in the past 24 hours prior to enrollment into the study. Given sildenafil’s half-life of 4 hours, 24 hours was deemed adequate to have negligible effects remaining of the drug. If possible, the dog was also fasted for eight hours to reduce gas within the gastrointestinal tract. Exclusion criteria included evidence of liver failure which was characterized by hypoalbuminemia, elevated liver enzymes, hypoglycemia, hypercholesterolemia, elevated bilirubin, and abnormal bile acids or ammonia tolerance. Additional exclusion criteria included known or suspected portosystemic shunts, portal vein obstructions, or if the patient is concurrently taking beta-blockers, nitrates, propranolol, amlodipine, or vasopressors. These disease processes and drugs were excluded as they cause changes to the portal vein pressures, velocity, and area.

**Imaging and measurements**

All images of the study were acquired using a Logiq S8 ultrasound machine (GE Healthcare, Wisconsin, USA) with a C3-10-D Broad Spectrum Micro-Convex (2-11 MHz) transducer (GE Healthcare, Wisconsin, USA). Each dog was placed in left lateral recumbency, and the ultrasound transducer was positioned on the right abdominal wall at the level of the 10th to 12th intercostal space. When necessary, a two-inch square was clipped on the right side of the patient at the 10th to 12th intercostal space on the dorsal one third of the ribs. Alcohol was applied to the skin and coupling gel was applied to the transducer prior to scanning. The aorta and portal vein were scanned while attempting to avoid renal parenchyma or pulmonary parenchyma in the footprint of the transducer. Attempts were made to acquire the measurements during quiet respiration. The area of the portal vein was obtained by acquiring a transverse image of the vessel (perpendicular to its long axis) and traced its outline. From this tracing, the area was
calculated by the ultrasound machine software. The same measurement was acquired for the aorta at the same level and the area calculated in the same manner.

Figure 2.1 Right lateral intercostal image of the porta hepatis.

Right lateral intercostal image of the porta hepatis. A denotes the aorta and is outlined with a dotted line that was traced on the ultrasound machine. B is the caudal vena cava. C is the portal vein and is outlined with a dotted line that is traced on the ultrasound machine. The relatively darker area above the caudal vena cava and portal vein is the liver.

The velocity of the portal vein was acquired using pulsed wave Doppler. The right branch of the portal vein was identified by moving the transducer one intercostal space cranially for most dogs. If the velocity at the right branch was unable to be obtained either due to small patient size or patient compliance, the velocity of the main portal vein was acquired at the same
level. The insonation angle was less than 60° for evaluation of the right branch of the portal vein in all scans. Angle correction was used as needed. The peak mean velocity was calculated using software the ultrasound machine.

Figure 2.2  Portal vein velocity

An ultrasound image of the portal vein and acquisition of the velocity using spectral Doppler from the right hepatic branch of the portal vein. PV stands for portal vein. The top portion of the image is the grey scale image of the liver and shows the location where the velocity measurement is obtained. The bottom half shows the measurement of the velocity of the right hepatic branch of the portal vein. The velocity measured the highest point which is 16.3 cm/s in this study which is normal. As the patient breathes, the vessel may fade in and out of the beam of the transducer, thus the reason the tracing becomes difficult to evaluate at -4 through -3. Additionally, notice the monophasic wave of the portal vein and the lack of sharp peaks or dips. The waveform is located above baseline, indicating that blood is flowing toward the transducer through the right hepatic branch.
The measurements were obtained prior to administration of sildenafil (time point 0), then 45 minutes, 90 minutes, and 120 minutes after administration.

**Statistical methods**

A paired t-test was used to determine if there is a significant change in velocity following administration of sildenafil. A pre-power analysis was performed and determined an optimum sample size of 25 dogs would allow the detection of a change of velocity of 3.4 cm/s (20% change assuming a mean of 17 cm/sec) or greater, a standard deviation of 5.79 cm/s, based off of the results of Sartor.\(^\text{30}\) An alpha of 0.05, a power of 0.80, and a two-tailed test was applied. Least squares means and 95% confidence intervals were utilized the lower and upper limits are reported for the portal vein velocity.

**Study results**

**Study population results**

Thirteen dogs were enrolled in the study. The average age was 9.4 years (range 2 – 16 years). Seven dogs were spayed females, four neutered males, and two intact males were enrolled. Dog were of various breeds and are listed in table 2.2. Body weight average was 16.2 kilograms (Kg) ranged from (2.5 to 29.5 Kg). All patients were being treated for pulmonary hypertension or heartworm disease.
Table 2.1  Study Population

<table>
<thead>
<tr>
<th>Breed</th>
<th>Gender</th>
<th>Weight (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yorkshire Terrier</td>
<td>FS</td>
<td>2.5</td>
</tr>
<tr>
<td>Labrador Retriever</td>
<td>FS</td>
<td>28.5</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>FS</td>
<td>5</td>
</tr>
<tr>
<td>King Charles Spaniel</td>
<td>MN</td>
<td>8.7</td>
</tr>
<tr>
<td>Standard Schnauzer</td>
<td>MN</td>
<td>12.7</td>
</tr>
<tr>
<td>Terrier mix</td>
<td>MN</td>
<td>11.9</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>FS</td>
<td>10</td>
</tr>
<tr>
<td>Mixed</td>
<td>MN</td>
<td>29.5</td>
</tr>
<tr>
<td>Shih Tzu</td>
<td>FS</td>
<td>4</td>
</tr>
<tr>
<td>Labrador cross</td>
<td>MI</td>
<td>38.2</td>
</tr>
<tr>
<td>Jack Russell Terrier</td>
<td>FS</td>
<td>7.3</td>
</tr>
<tr>
<td>Mixed</td>
<td>FS</td>
<td>16.6</td>
</tr>
<tr>
<td>Mixed</td>
<td>MI</td>
<td>35.5</td>
</tr>
</tbody>
</table>

Breed, gender, and weight of enrolled patients. FS: spayed female, MN: male neutered male, MI: male intact. Weight is reported in kilograms.

**Imaging results**

Acquisition of the aorta and portal vein was feasible in all patients. Gas within the duodenum was occasionally present, but did not preclude vascular evaluation. Table 2.3 provides the average results for all time points studied for the portal vein area, aorta area, and portal vein to aorta area ratio. A total of 64 data points was acquired during the study period. One 90-minute time point data was lost.

Table 2.2  Average portal vein area, aorta, and portal vein area to aorta ratio

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Portal vein area (cm²)</th>
<th>Aorta area (cm²)</th>
<th>Portal vein/ aorta ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.36(0.27-0.46)</td>
<td>0.78(0.52-1.05)</td>
<td>0.49(0.3-0.69)</td>
</tr>
<tr>
<td>45</td>
<td>0.36(0.27-0.45)</td>
<td>0.81(0.54-1.07)</td>
<td>0.45(0.26-0.65)</td>
</tr>
<tr>
<td>90</td>
<td>0.32(0.22-0.41)</td>
<td>0.80(0.54-1.07)</td>
<td>0.41(0.22-0.6)</td>
</tr>
<tr>
<td>120</td>
<td>0.32(0.22-0.41)</td>
<td>0.78(0.51-1.04)</td>
<td>0.47(0.27-0.66)</td>
</tr>
</tbody>
</table>

Average portal vein area, aorta area, and portal vein to aorta area ratio for each time examined in 13 dogs. The values provided are the average for the examined time ± the standard error.
No statistically significant differences were identified in the portal vein area, aorta area, or portal vein to aorta area ratio over time.

Table 2.3 Average portal vein velocity and congestion index over time

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Portal vein velocity (cm/s)</th>
<th>Congestion index</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.36(9.95-15.36)</td>
<td>0.029(0.022-0.037)</td>
</tr>
<tr>
<td>45</td>
<td>11.96(9.63-14.87)</td>
<td>0.030(0.023-0.038)</td>
</tr>
<tr>
<td>90</td>
<td>11.30(9.07-14.07)</td>
<td>0.028(0.020-0.035)</td>
</tr>
<tr>
<td>120</td>
<td>10.40(8.37-12.92)</td>
<td>0.030(0.023-0.038)</td>
</tr>
</tbody>
</table>

Portal vein velocity and congestion index and 95% confidence intervals.

No statistically significant differences were detected at each time point for the portal vein and congestion index.

Discussion

Conclusions

The hypothesis that sildenafil will cause an increase of the portal vein area, portal vein to aorta area ratio, portal vein velocity and decrease congestion index in the normal dog was rejected. The results of this study showed there was no statistically significant acute changes of the portal vein parameters following oral administration of sildenafil at any measured timepoint. Although not statistically significant, on average the portal vein velocity was progressively lower at each subsequent evaluation. The findings of this study are interesting given the body of knowledge available. In the study performed by Colle et al. sildenafil caused increase in mesenteric blood flow, portal vein pressure, and decreased the mean mesenteric arterial pressure in both the control rats, and the experimental rats with a ligated common bile duct. It is interesting to note, that the experimental group had a smaller effect than the control group when sildenafil was introduced. Similar conflicting findings were reported in human literature with
sildenafil causing an increase and decrease of the portal venous pressures.\textsuperscript{19-21} It is possible that sildenafil may cause a mild increase in portal vein pressure in the canine patient by increasing the mesenteric arterial blood flow as with previously reported studies. This theory cannot be confirmed with this study and may need further and higher-powered investigation. In the aforementioned studies, patients evaluated had an altered hemodynamic state which likely played a large part of the findings. In the normal patient, the buffer system of the liver becomes activated when there is an alteration in the blood flow to the liver from the portal vein. Minor changes, such as those caused by sildenafil, are likely able to be overcome by this system and mild to no effects would be seen. There are numerous causes of portal vein hypertension such as prehepatic, hepatic, and post hepatic causes discussed above. Further investigation may help identify specific diseases and specific times when sildenafil may be beneficial for each scenario.

The congestion index is one of the more accurate ways to diagnose portal vein hypertension since it incorporates velocity and area. In this study, the results were deemed normal and did not change with the differences in time.

In this study, the portal vein velocities on the low end of normal and did not change significantly with the administration of sildenafil. At the authors institution, the described right intercostal approach is frequently employed, but anecdotally, the values are slightly low. This is likely due to the innate challenges of measuring the right hepatic branch of the portal vein velocity and patient compliance.

Prior studies have previously used the diameter for the portal vein to aorta ratios. For this study, the built-in software of the ultrasound machine was utilized to calculate the area. Cross sectional area was chosen as most calculations, such as congestion index, utilize the area rather than just the diameter. Furthermore, cross sectional area may be more accurate since vessels may
not always take on a perfect circular shape in vivo. Therefore, the values obtained are not directly comparable to the reported normal values, but used as an additional tool to monitor over time and potential changes the portal system may have had. Additionally, this study may serve as a normal ratio for the portal vein to aorta cross sectional area.

There are three possible explanations for the results of this study. The first possibility is that sildenafil does not have a significant effect on the portal vein. Despite PDE-5 receptors being present on the vascular endothelium, receptors may be in a lower concentration within the portal veins. Additionally, as sildenafil causes a decrease in the NO effects, the portal vascular system may not respond as intensely to its effects as the pulmonary vascular system does.

The second conclusion to consider is that the effects of the buffer system may be strong enough to suppress the acute changes from sildenafil. Pressure changes within the portal vein cause changes in the hepatic arteries, thus leading to a relatively constant blood supply to the liver. A reflex of the buffer system in times of low hepatic arterial pressure may lead to portal system hypertension. Since these vessels converge within the hepatic parenchyma at the sinusoids, the hepatic resistance may then increase, leading to increased portal pressure and decreased velocity. Given relatively no changes are seen within the measured parameters at any time evaluated, this conclusion is considered less likely as a decrease in portal vein velocity with a reflex change would be expected to be observed with the multiple time points measured.

Finally, all patients in this study did were free of evidence of hepatic disease. Therefore, the effects of sildenafil may have minimal effects on normal patients, but patients with a compromised circulatory system may experience more significant and observable changes.
Limitations

Ultrasound itself proves to be a difficult modality with which to evaluate portal vein velocity due to the inherent differences between different operators. This was combated by having the same sonographer perform the study at every time point for each dog leading to minimal operator variance. Although this may limit the differences, variables due to the patient such as gas within the intestines, patient anatomy, and patient compliance can prove to be difficult.

During the study, the angle of insonation likely varied slightly between scans, which may introduce error in the measurements. For this reason, the insonation angle was kept as low as possible by attempting to measure the portal vein velocity at the right hepatic branch of the portal vein. When visible, the right hepatic branch is angled towards the ultrasound transducer, thus being an ideal area to obtain the portal vein velocity. Although this was attempted to be constant and as low as possible, it still introduced some degree of error in the velocity measurements. Although the right hepatic branch of the portal vein may be an optimal anatomic region to interrogate the velocity of the portal vein, it can be challenging to image. This region moves with respiration and can yield inaccurate readings.

Variations in dog size may cause error when comparing results between dogs. To correct for this variability, each dog served as its own control with data obtained both before and after the administration of sildenafil. The portal vein velocity can be slightly phasic and the velocity is slightly altered with respiration. This effect was minimalized by making measurement attempts during quiet respiration.

The main limitation of this study was the low number of enrolled patients potentially contributing to a Type II error. It is possible that a higher-powered study may reveal changes that
this study was unable to demonstrate. With that in mind, trends were not identified statistically in the data, and further enrollment of dogs was deemed unlikely to yield a difference.

**Future studies**

This study can also be performed in dogs that have evidence of hepatic disease. Dogs with hepatic disease may already have compromised the buffer system and changes of the portal vasculature may have a more significant effect on these patients. Given the discrepancy of response to sildenafil in human medicine, careful patient selection should be considered when considering adding sildenafil to these patients and may be best reserved for patients in right sided heart failure leading to portal vein hypertension.

Additionally, body condition was not evaluated in this study. Given that obese patients tend to have an increased congestion index, this study could be performed with body condition score controlled and evaluate the effects sildenafil may have on this set of patients.

**Synopsis**

This study found no significant difference in the portal vein and aorta areas, portal vein velocity, or the congestion index within two hours of the administration of sildenafil in healthy dogs.
REFERENCES


