Effect of Warmed and Humidified Carbon Dioxide for Pneumoperitoneum on Core Body Temperature, Cardiorespiratory Variables, Thromboelastography, Systemic Inflammation, Peritoneal Response, and Postoperative Pain during Laparoscopy in Healthy Mature Dogs

by
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ABSTRACT

EFFECT OF WARMED AND HUMIDIFIED CARBON DIOXIDE FOR PNEUMOPERITONEUM ON CORE BODY TEMPERATURE, CARDIORESPIRATORY VARIABLES, THROMBOELASTOGRAPHY, SYSTEMIC INFLAMMATION, PERITONEAL RESPONSE, AND POSTOPERATIVE PAIN DURING LAPAROSCOPY IN HEALTHY MATURE DOGS.

Dr. Jacqueline Scott  
Advisor: Dr. Ameet Singh  
University of Guelph, 2018

Laparoscopy with standard CO₂ (STCO₂) insufflation has been postulated to contribute to perioperative hypothermia, cardiorespiratory alterations, peritoneal irritation, and postoperative pain. Hypothermia has numerous detrimental complications including organ failure, immunosuppression, prolonged recovery, and coagulopathy. The objective of this research was to evaluate the effect of warmed, humidified carbon dioxide (WHCO₂) pneumoperitoneum on core body temperature, cardiorespiratory variables, thromboelastography, systemic inflammation, peritoneal response, and postoperative pain in healthy, mature, dogs undergoing laparoscopy. No clinically significant differences were reported in any of the variables measured. These data suggest the use of WHCO₂ for pneumoperitoneum in healthy, mature, dogs undergoing laparoscopy did not differ from the use of STCO₂.
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A special thank you to my partner James for holding my hand and walking in the snow with me. Finally thank you to all my friends and family for all your emotional and financial support in this crazy journey. Without your support this dream would never have been possible.
DECLARATION OF THE WORK PERFORMED

I declare that with the exception of the items below, all work reported in this thesis was performed by me.

Statistical analysis was performed by William Sears and Gabrielle Monteith, of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

Pain scores were assigned by a blinded research participant Jessica Kilkenny, of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

The ELISA for C-reactive protein and IL-6 serum concentration levels were performed by Michelle Beaudoin-Kimble, of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph.

Histopathology and scanning electron microscopy tissue samples were processed and evaluated by a board certified pathologist, Dr. Robert Foster, of the Department of Pathobiology, Ontario Veterinary College, University of Guelph.

I, Jacqueline Scott performed all the writing, graphing and tablet formatting in this thesis with editorial comments made by Dr. Ameet Singh, Dr. Alex Valverde, Dr. Shauna Blois and Dr. Alex zur Linden, of the Department of Clinical Studies, Ontario Veterinary College, University of Guelph.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CO</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CVP</td>
<td>Central venous pressure</td>
</tr>
<tr>
<td>DAP</td>
<td>Diastolic arterial blood pressure</td>
</tr>
<tr>
<td>ET&lt;sub&gt;CO₂&lt;/sub&gt;</td>
<td>End-tidal CO₂</td>
</tr>
<tr>
<td>ET&lt;sub&gt;ISO&lt;/sub&gt;</td>
<td>End-tidal isoflurane concentration</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>LiDCO</td>
<td>Lithium dilution cardiac output</td>
</tr>
<tr>
<td>MA</td>
<td>Maximum amplitude of blood clot</td>
</tr>
<tr>
<td>MAC</td>
<td>Minimum alveolar concentration</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial blood pressure</td>
</tr>
<tr>
<td>SAP</td>
<td>Systolic arterial blood pressure</td>
</tr>
<tr>
<td>STCO₂</td>
<td>Standard temperature carbon dioxide</td>
</tr>
<tr>
<td>TEG</td>
<td>Thromboelastography</td>
</tr>
<tr>
<td>WHCO₂</td>
<td>Warm humidified carbon dioxide</td>
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CHAPTER I: Literature Review

1.1 - Laparoscopy using standard carbon dioxide (STCO₂) insufflation

Laparoscopic instruments for examining human orifices date back to 1805, and was pioneered by gynecologists in the 1960s and 70s, however it was not until the 1980 that laparoscopic surgery started to be more widely accepted.¹ Laparoscopic surgery has been found by multiple systematic reviews to reduce postoperative discomfort, and shorten hospitalization times in people.²–⁴ Today laparoscopic surgery is considered the gold standard for many procedures, with over 500,000 laparoscopic cholecystectomies performed annually in the United States alone.⁵

Laparoscopy uses a camera and other instruments introduced into the abdomen through small incisions in order to perform minimally invasive surgery. A working space is created within the abdomen, most commonly by using carbon dioxide (CO₂) insufflation. Carbon dioxide is transferred from gas tanks towards the patient through a mechanical insufflator via a hose, and introduced into the peritoneal cavity through a cannula. The mechanical insufflator allows monitoring and maintenance of the intra-abdominal pressure (IAP) at recommended values, usually around 10 mm Hg. This pressure provides adequate working space, while maintaining physiologically acceptable cardiorespiratory depression.⁶ Carbon dioxide is the most commonly used gas for insufflation as it is cheap, noncombustible, colorless, excreted by the lungs and highly soluble in water, reducing the risk of gas embolism.⁷

The development of minimally invasive surgery (MIS) in veterinary medicine parallels the human field however with a 20 year delay.⁸ Initially developed as a diagnostic tool, the practice of minimally invasive surgery has grown rapidly in the last 15 years in veterinary medicine.⁹–¹⁴ In a 2010 survey by Bleedorn et al., 1216 surgery diplomates were survey on their current MIS use in practice.¹⁵ Eighty-six percent of small animal diplomates performed minimally invasive surgery with a steady increase in use over time documented. Less than 20 of the diplomates who qualified prior to 1985 were using MIS compared to almost 120 who qualified in the last 4 years prior to publication.¹⁵
1.1.1 - Current use and indications of STCO₂ laparoscopy in veterinary medicine

Laparoscopy is indicated in a variety of small animal conditions, from liver biopsies, prophylactic gastropexy, ovariohysterectomy to more advanced procedures such as cholecystectomy, adrenalectomy, or ureteronephrectomy. Distinct advantages over a traditional “open” approach have been reported in the veterinary literature with a reduction in pain, a more rapid return to function, lower surgical site infection rate, and reduced hospitalization time.

1.1.2 - Detrimental effects of STCO₂ insufflation

While CO₂ is the most common gas used for insufflation, it has been suggested that capnoperitoneum can lead to detrimental effects on cellular, metabolic, cardiovascular and respiratory physiology. Carbon dioxide is a known irritant to the peritoneal surface due to the release of inflammatory mediators, which are associated with increased pain and adhesion formation. The systemic acidosis and hypercapnia observed due to CO₂ absorption through the peritoneal surface can have vasodilatory effects on the blood vessels, lead to myocardial depression and indirect sympathetic stimulation. An increase in the IAP due to the establishment of a pneumoperitoneum reduces venous return and increases systemic vascular resistance leading to reduced cardiac output.

Increases in IAP limit diaphragm excursion, reduce pulmonary compliance, functional residual capacity, and vital capacity of the lung. There is an increase in end tidal CO₂ and an inability to compensate for this elevation, which is exacerbated by the absorption of insufflated STCO₂ leading to fatigue and the need for mechanical ventilation.

It has also been hypothesized that laparoscopic surgery using STCO₂ insufflation exacerbates perioperative hypothermia. The mechanism explaining this theory is based on heat loss from convection and evaporative cooling. Convection is energy transfer from the warm abdominal cavity and is proportional to the rate of gas flow, specific heat capacity of the gas and the temperature of the gas used. The average CO₂ gas flow in a human patient varies considerably (12 - 801L) and is related to the volume of CO₂ leaking around port sites, the duration and type of procedure, and the number of
instrument changes.\textsuperscript{31} Evaporation contributes the most to heat loss during laparoscopy with saturation of dry insufflated CO\textsubscript{2} gas particles using heat energy.\textsuperscript{30}

These detrimental effects have lead to the development of alternative methods of creating a working space within the peritoneal cavity.

1.1.3 - Alternatives to STCO\textsubscript{2} insufflation

Helium insufflation has been proposed to produce less cardiorespiratory and intra-peritoneal immunologic changes compared to STCO\textsubscript{2}.\textsuperscript{7,32} In 20 human patients who had a laparoscopic cholecystectomy, it was found to preserve pH and PaCO\textsubscript{2} in procedures over 40 - 60 minutes in duration, however protracted subcutaneous emphysema was noted in 15\% of patients, taking several weeks to resolve.\textsuperscript{33} This is because helium is less soluble than CO\textsubscript{2} and more serious potential complications include an increased risk of fatal venous gas embolism.\textsuperscript{7}

Nitrous Oxide was the preferred gas in the 1970s but its use for insufflation during laparoscopy declined due to concerns for combustibility limiting its use with electrocautery devices.\textsuperscript{7}

While the use of alternative gases may combat the concern of systemic acidosis and hypercapnia, the cardiorespiratory effects seen in pneumoperitoneum are primarily related to increases in the IAP.\textsuperscript{34} Lift laparoscopy, or gasless laparoscopy has been suggested as an alternative to minimize the cardiorespiratory concerns and has also been hypothesized to reduce perioperative hypothermia.\textsuperscript{23,34,35} Fransson et al. (2014) found no significant difference in cardiorespiratory variables in dogs undergoing lift laparoscopy compared to STCO\textsubscript{2} insufflation for ovariohysterectomy.\textsuperscript{36} Interestingly this study also found no significant difference in the degree of pain experienced between each treatment group, suggesting that stretching of the peritoneal tissues is responsible for the postoperative discomfort rather than the capnoperitoneum.\textsuperscript{36} In a human randomized clinical trial, the tympanic membrane temperature decreased by 0.2\textdegree C during a 107 minute surgical procedure using STCO\textsubscript{2} insufflation compared to lift laparoscopy.\textsuperscript{37}

The insufflation of warmed and humidified CO\textsubscript{2} has been suggested as a alternative to STCO\textsubscript{2} in order to create a pneumoperitoneum environment that preserves cellular and metabolic function, while also preventing hypothermia.\textsuperscript{38,39}
1.2 - Perioperative hypothermia

Heat production in the body is due to metabolism and is regulated by thermoreceptors in the hypothalamus that initiate reflexes to increase heat production and reduce heat loss.\textsuperscript{40,41} Thermoregulation normally has a very narrow set point of +/- 0.2°C, however anesthetic drugs can increase the interthreshold range by approximately 3.5°C.\textsuperscript{42} General anesthesia induces hypothermia in two stages. First, heat is rapidly redistributed from the core, out to the periphery within the first 60 minutes. This redistribution is because of reduction in the temperature threshold in the thermoregulatory center and vasodilation. In the second stage, general anesthesia reduces heat production due to a lowered metabolic rate, while surgery increases heat loss from radiation, conduction, convection and evaporation.\textsuperscript{42,43} Radiation has been identified as the most important cause of perianesthetic heat loss and unfortunately is not inhibited by traditional methods to prevent hypothermia (warmed water blankets, forced warmed air heaters).\textsuperscript{42}

1.2.1 - Prevalence of hypothermia in veterinary patients

Perioperative hypothermia in dogs is defined as a body temperature < 36.5°C.\textsuperscript{44} Inadvertent perianesthetic hypothermia is one of the most common complications of anesthesia,\textsuperscript{40} with 83.6% of dogs undergoing general anesthesia for surgical or diagnostic procedures, classified as hypothermic at the time of anesthetic recovery.\textsuperscript{44} In a retrospective study evaluating hypothermia in >1500 dogs, 51.5% of patients were considered slightly hypothermic with a core body temperature between 38.5°C and 36.5°C, 29.3% were moderately hypothermic between 36.5°C and 34°C, and 2.8% severely hypothermic with a body temperature below 34°C.\textsuperscript{44} Factors that can influence perioperative hypothermia include operating suite air temperature, an open body cavity, direct contact with surgical steel tables, surgical scrub, and lavage.\textsuperscript{40}

1.2.2 - Complications associated with hypothermia

Hypothermia has numerous detrimental effects leading to an increase in patient morbidity.\textsuperscript{40,41,45–53}
Table 1.1 - Complications associated with hypothermia

<table>
<thead>
<tr>
<th>Complications</th>
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<tbody>
<tr>
<td>Organ dysfunction</td>
<td>Lenhardt, Marker, Goll et al. 1997</td>
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<td></td>
<td>Frank, Fleisher, Breslow et al. 1997</td>
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<tr>
<td></td>
<td>Armstrong &amp; Roberts 2005</td>
</tr>
<tr>
<td></td>
<td>Clark-Price 2015</td>
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<tr>
<td>Coagulopathy/ Increased transfusion requirements</td>
<td>Schmeid, Kurz, Sessler et al. 1996</td>
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<tr>
<td></td>
<td>Lenhardt, Marker, Goll et al. 1997</td>
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<tr>
<td></td>
<td>Kettner, Sitzwohl, Zimpfer et al. 2003</td>
</tr>
<tr>
<td></td>
<td>Rundgren &amp; Engstrom 2008</td>
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<tr>
<td></td>
<td>Clark-Price 2015</td>
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<tr>
<td>Impaired humoral and cellular immunity</td>
<td>Kurz, Sessler, Lenhardt 1996</td>
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<tr>
<td></td>
<td>Lenhardt, Marker, Goll et al. 1997</td>
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<tr>
<td></td>
<td>Beilin, Shavit, Razumovsky et al. 1998</td>
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<tr>
<td></td>
<td>Armstrong &amp; Roberts 2005</td>
</tr>
<tr>
<td></td>
<td>Beal, Brown, Shofer 2009</td>
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<tr>
<td></td>
<td>Clark-Price 2015</td>
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<tr>
<td>Prolonged recovery</td>
<td>Kurz, Sessler, Lenhardt 1996</td>
</tr>
<tr>
<td></td>
<td>Lenhardt, Marker, Goll et al. 1997</td>
</tr>
<tr>
<td></td>
<td>Pottie, Dart, Perkins et al. 2007</td>
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<td></td>
<td>Clark-Price 2015</td>
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Hypothermia impairs platelet function by impairing the release of thromboxane A2, which is necessary for the formation of the platelet plug. Hypothermia also impairs the function of enzymes in the coagulation cascade. Coagulation impairments demonstrated with thromboelastography (TEG) in the hypothermic patient have been well established in human patients. In a meta-analysis of fourteen studies Rajagopalan et al. (2008) found even mild hypothermia of <1°C increases blood loss by 16% and the relative risk for transfusion by 22%. 
Hypothermia induced vasoconstriction can lead to hypoxia of local tissues and impaired immunological responses reducing patient resistance to infection.\textsuperscript{41} Human patients undergoing colorectal surgery with mean postoperative temperatures of 34.7°C had infection rates 3 times as high as those patients with postoperative temperatures of 36.6°C.\textsuperscript{53} Conversely, a retrospective veterinary study showed no increase in the infection rate in clean surgical wounds of dogs with mild perioperative hypothermia 36.4°C.\textsuperscript{47}

Prolonged recovery and hospitalization is identified in patients who suffer from perioperative hypothermia.\textsuperscript{45,47} In a study involving 200 human patients who underwent colorectal surgery, those that were actively warmed with forced air blankets had a final core temperature 2°C higher than those that had only passive warming.\textsuperscript{53} Consequently these normothermic patients had a shorter duration of hospitalization by 2.6 days compared to the hypothermic treatment group ($P = 0.01$).\textsuperscript{53}

**1.2.3 - Prevention of hypothermia**

It is standard practice to use circulating water blanket, forced-air and warming panels in order to prevent hypothermia in the surgical patient.\textsuperscript{40} Warmed forced air is considered the most effective thermal management in dogs and cats.\textsuperscript{55,56} Warmed sterile saline lavage at 43°C for 2-6 minutes is also recommended when an open approach is used.\textsuperscript{57}

Warmed and humidified CO\textsubscript{2} insufflation is postulated as a measure in which laparoscopic hypothermia could be reduced by minimizing heat losses.\textsuperscript{38} Warming and humidification of insufflated CO\textsubscript{2} to 37°C and 97% relative humidity is hypothesized to minimize heat loss from evaporation as the gas particles are already saturated prior to introduction within the peritoneal cavity.\textsuperscript{38} It would also decrease convection heat transfer between the abdominal organs and gas particles compared to standard room temperature CO\textsubscript{2} which is reportedly ~21°C.\textsuperscript{58} Binda et al. (2004) reported that laparoscopic hypothermia is most likely due to the energy required to humidify insufflated gas, as it requires 577 cal to vaporize 1 g of water compared to only 0.00003cal to heat 1 ml of CO\textsubscript{2} by 1°C.\textsuperscript{59}
1.3 - Cardiorespiratory variables in laparoscopy

The physiological changes noted in laparoscopy relate not only to the increased intra-abdominal pressure created by a pneumoperitoneum but also to the systemic absorption of CO₂.\textsuperscript{28,60,61} While the insufflation of the abdomen causes compression of thin walled vessels, affecting blood flow and pressures, hypercapnia also causes myocardial depression and vasodilation resulting in sympathetic stimulation.\textsuperscript{61} As discussed above in “Detrimental effects of STCO\textsubscript{2} insufflation”, alterations in almost all cardiorespiratory variables are reported including heart rate, cardiac output, stroke volume, inferior vena cava flow, systemic vascular resistance, arterial partial pressure of oxygen, oxygen delivery, mean arterial pressure, arterial partial pressure of CO\textsubscript{2}, pH, pulmonary compliance, and minute ventilation.\textsuperscript{24,27,28} While it is generally considered these alterations are transient, their effects may be of importance in the cardiac or respiratory compromised patient.\textsuperscript{26}

1.3.1 - Cardiac output

It is well established pneumoperitoneum alters venous return due to compression of the vena cava.\textsuperscript{43,62} Venous return is the measure of blood returning from the body to the heart to fill in diastole.\textsuperscript{63} An increase in the end diastolic volume results in an increase in stroke volume, according to the Frank-Starling law.\textsuperscript{63} Stroke volume and heart rate are contributors to cardiac output, which is a measure of the amount of blood pumped by the heart per minute.\textsuperscript{64} Cardiac output is an important variable to determine sufficient blood flow and oxygenation to vital organs.\textsuperscript{64} Cardiac output can be measured by a variety of methods including direct/indirect fick, dilution methods (thermodilution, lithium dilution, dye dilution), arterial pulse contour analysis, heat transfer, electrical impedance cardiography, CO\textsubscript{2} rebreathing, and transesophageal doppler.\textsuperscript{64}

Dilutional techniques to measure cardiac output involve the intravenous administration of a solution and the measurement of changes in blood temperature or in the concentration of the solution in arterial blood.\textsuperscript{65,66} Lithium dilution has been validated
against the “gold standard” technique of thermodilution and offers advantages as it eliminates the need for pulmonary arterial catheterization.\textsuperscript{65,67,68}

When pneumoperitoneum is established at an IAP of \textasciitilde10 mm Hg, cardiac output is often preserved despite a fall in stroke volume, due to a concurrent increase in heart rate.\textsuperscript{27,28,69,70} Mayhew et al. (2013) evaluated working space and cardiorespiratory variables at different insufflation pressures in cats.\textsuperscript{6} No significant increase in working space was gained above 8 mm Hg, while a significant increase in PaCO\textsubscript{2}, pH and SVI was noted at 15 mm Hg.\textsuperscript{6}

**1.3.2 - Stroke volume**

Stroke volume is the volume of blood ejected by the left ventricle per beat and is determined by preload, afterload and contractility. Alterations in any factors that contribute to these can affect stroke volume.\textsuperscript{63} Pneumoperitoneum effects stroke volume by reducing preload and increasing afterload.\textsuperscript{26} A decrease of 15-80\% has been reported in the research and clinical veterinary literature, with intra-abdominal pressures ranging from 6 - 30 mm Hg.\textsuperscript{27,28,62,70}

**1.3.3 - Systemic vascular resistance**

Systemic vascular resistance is the resistance which must be overcome in order for blood to flow and is controlled by vasomotor tone.\textsuperscript{64} An overall increase in systemic vascular resistance is reported in veterinary laparoscopy despite the vasodilatory effect of hypercapnia due to the compressive effects of increased IAP.\textsuperscript{6,69,71} Some reports however find this less consistent. Ho et al. (1995) found no significant increase in total peripheral resistance in research pigs until intra-abdominal pressures of over 20 mm Hg were applied,\textsuperscript{70} and Duke et al. (1996) reported a trend in dogs towards elevation over 180 minutes of insufflation, but no significant difference was seen.\textsuperscript{27}

**1.3.4 - Oxygen delivery, consumption and extraction ratio**

The literature reports minimal changes to oxygen delivery, consumption, and extraction ratio in animals undergoing laparoscopic surgery compared to traditional open
techniques.\textsuperscript{6,27,70} Mayhew et al. (2013) reported an increase in oxygen delivery after 30 minutes at 15 mm Hg compared to 4 mm Hg in cats.\textsuperscript{6} Oxygen delivery is the product of cardiac output and arterial oxygen concentration, therefore this increase may have been related to the reported increase in cardiac index seen in this study.\textsuperscript{6}

\textbf{1.3.5 - Arterial blood pressure}

Arterial blood pressure is expected to increase due to sympathetic stimulation and increased intra-abdominal pressures.\textsuperscript{61,70,72} Several studies have however, reported stable arterial blood pressure,\textsuperscript{19,62,71} and while blood pressure is often used as a convenient surrogate to blood flow, cardiovascular studies have found no correlation between blood pressure and cardiac output.\textsuperscript{73,74} Williams et al. (1993) found insignificant changes in mean arterial blood pressure in 6 dogs undergoing pneumoperitoneum at 15 mm Hg and 30 mm Hg, in Trendelenburg and reverse Trendelenburg positions.\textsuperscript{62} The authors concluded that alterations in cardiac output and stroke volume were not accompanied by nor could they detect changes in mean arterial pressure.\textsuperscript{62}

\textbf{1.3.6 - Arterial partial pressure of CO$_2$ and pH}

As CO$_2$ is absorbed across the peritoneal surface, systemic PaCO$_2$ increases with a concurrent decline in pH.\textsuperscript{19,69,70,75} Severe hypercapnia (55-70 mm Hg) can lead to elevations in heart rate, systolic blood pressure, central venous pressure, cardiac output, stroke volume, and a decrease in peripheral vascular resistance.\textsuperscript{76} However mild hypercapnia (45-50 mm Hg) as is seen in clinical practice owing to adjustment of minute ventilation, appears to have little impact on hemodynamic function \textsuperscript{76}.

\textbf{1.4 - Coagulation variables in laparoscopy}

The hemostatic pathway is complex with many interacting factors.\textsuperscript{77} The traditional cascade model has been replaced with the cell-based model, which is more accurate of the hemostatic process in vivo.\textsuperscript{77} The cell-based model relies on initiation of coagulation due to exposure of extravascular tissue factor-bearing cells to plasma.\textsuperscript{78}
Coagulation undergoes amplification with platelet activation. These activated platelets provide the surface for propagation and large scale thrombin generation.\textsuperscript{77,78}

Coagulation can be affected by surgical stimulation, with tissue trauma exposing tissue factor bearing cells and inflammatory cytokines upregulating von Willebrand factor expression, increasing plasminogen activator inhibitors, and decreasing tissue plasminogen activation, anti-thrombin, and activated protein C.\textsuperscript{79} It is currently unknown how STCO\textsubscript{2} insufflation influences hemostasis compared to traditional open techniques, however the author of this thesis theorize that the peritoneal irritation and perioperative hypothermia seen with STCO\textsubscript{2} may lead to changes in hemodynamic physiology.

1.4.1 - Hemostatic testing

Routinely reported hemostatic testing in veterinary patients includes platelet count, buccal mucosal bleeding times, platelet function testing (more limited availability), prothrombin time, activated partial thromboplastin time, activated clotting time, concentration of fibrin split products, d-Dimers, and fibrinogen.\textsuperscript{77} These tests are limited in that they assess only one point in the coagulation pathway. A global assessment of coagulation can be made using viscoelastic testing such as Thromboelastography (TEG).\textsuperscript{77}

Thromboelastography evaluates clot formation from initiation through amplification and propagation, to fibrinolysis.\textsuperscript{80} It can demonstrate the strength and stability of the clot as well as the dynamics of its breakdown. As such TEG more closely resembles the in vivo hemostatic process than other clinically available hemostatic tests.\textsuperscript{77} Recalcified, citrated blood is added to a plastic cup, which is oscillated around a pin. As fibrin forms between the cup and the pin a transducer records the torque on the pin as it starts to move with the cup.\textsuperscript{77}
Figure 1.1 - Normal Thromboelastography trace.

The reaction time (R) is the time interval between the start of coagulation and the point where the clot reaches 2-mm in amplitude. The clotting time (K) is the time for clot formation to a defined clot amplitude of 20 mm. The alpha angle is the rapidity of fibrin cross-linking and the maximum amplitude (MA) is a measure of clot strength. Fibrinolysis (LY30) is reflected as a percentage decrease in the amplitude 30 minutes after MA.⁸¹

1.5 - Inflammatory biomarkers in soft tissue surgery

Surgical stimulus can result in inflammation of tissues.⁷⁹ In the acute inflammatory response there is vasodilation, an increase in vascular permeability and leukocyte extravasation leading to an influx of inflammatory mediators and acute phase proteins.⁷⁹ Pro-inflammatory cytokines TNF-α, IL-1β, IL-6 are released from macrophages, which in turn alter the synthesis of acute phase proteins in the liver. Positive acute phase proteins increase in plasma concentration by 25% during inflammation and include C-reactive protein, serum amyloid A, serum amyloid P, complement proteins, coagulation factors, and kallikrein-kinin proteins. These cytokines and acute phase proteins can be measured in the serum as biomarkers for inflammation.⁷⁹,⁸²
1.5.1 - C-reactive protein

C-reactive protein (CRP) promotes complement activation, regulates leukocyte infiltration, inhibits respiratory burst in neutrophils, and activates macrophages to produce TNF-α, IL-1β, IL-6 and tissue factor.\textsuperscript{79,82} C-reactive protein is a major acute phase protein in dogs and is used as a diagnostic test for systemic inflammation with quantitative measurement of canine CRP concentrations.\textsuperscript{82-84} While a single measure of CRP is of limited value, elevation in CRP concentrations has been evaluated as a predictive measure of systemic inflammatory response syndrome and morbidity following soft tissue surgery.\textsuperscript{83,85} A decreasing CRP concentration from admission within 48 hours, has been found to predict survival in 94% of dogs with systemic inflammatory syndrome or sepsis.\textsuperscript{86}

1.5.2 - Interleukin-6

Interleukin-6 (IL-6) is produced by macrophages, T-cells, epithelial cells, and enterocytes. It initiates hepatic synthesis of acute phase proteins and influences proliferation of lymphocytes.\textsuperscript{79} It has been shown to increase proportionately to the duration and severity of the condition in human patients,\textsuperscript{87} and has been validated in dogs as a valuable predictor of sepsis and systemic inflammatory response syndrome (SIRS).\textsuperscript{88,89} In a retrospective study involving 69 dogs survivors from a range of severe diseases in the intensive care unit were found to have a significantly lower median serum IL-6 concentrations compared to non-survivors.\textsuperscript{89} In a earlier study elevations in IL-6 concentrations at admission were correlated with more severe disease, increased mortality rate and earlier fatality in dogs with systemic inflammatory syndrome and sepsis.\textsuperscript{88} These findings are in contrast to those of Fransson et al. (2006) who reported significant overlap in the plasma concentration of IL-6 between healthy and diseased dogs.\textsuperscript{85}

1.5.3 - Inflammatory biomarkers in laparoscopy

The use of biomarkers in human medicine to evaluate the postoperative inflammation has proven minimally invasive surgery to have a reduced inflammatory
response compared to traditional open techniques as measured by IL-6 and CRP. In humans, laparoscopic cholecystectomy is a common procedure performed for symptomatic cholecystolithiasis. Laparoscopic cholecystectomy has been associated with a significantly shorter hospital stay, and shorter convalescence compared with traditional open cholecystectomy. In human patients undergoing laparoscopic cholecystectomy, peak IL-6 concentrations have been found to be significantly lower (51 pg/mL) than patients who underwent open cholecystectomy (124 pg/mL). Researchers concluded this finding supports the true minimally invasive nature of laparoscopic cholecystectomy. This same study also evaluated CRP as a confirmatory test and reported similar results with significantly lower concentrations in patients who had a laparoscopic procedure (24 mg/L) compared to an open approach (104 mg/L).

1.6 - Peritoneal response to STCO\textsubscript{2} insufflation

The peritoneum is a serous membrane surface comprised of squamous epithelial cells and is easily injured. Its primary function is to reduce friction within the abdominal cavity. It has been found the insufflation of the peritoneal cavity with CO\textsubscript{2} alters peritoneal morphology, pH, and the local inflammatory response.

1.6.1 - Peritoneal morphology

Evaluation of peritoneal health is best performed using scanning electron microscopy due to the small size of the mesothelial cells. Scanning electron microscopy uses electrons rather than light as the source of illumination. A primary electron beam is directed towards the surface of interest, causing excitement of different energy signals, which are detected and transmitted into photons which ultimately generates a image.

When the peritoneum is exposed to CO\textsubscript{2} gas, retraction of mesothelial cells is seen with exposure of the underlying basal lamina. Mice exposed to a capnoperitoneum at an IAP of 6 mm Hg for 30 minutes had the peritoneal surface evaluated with scanning electron microscopy. No alterations in peritoneal morphology were seen intra-operatively, however progressive changes with widening of intercellular
clefts and exposure of the basal lamina was evident in the first 12 hours postoperatively. Mesothelial regeneration was noted over the remaining 84 hours and a nearly confluent layer of microvilli-covered cells were seen at 96 hours postoperatively.\textsuperscript{106}

This denuded basal lamina is thought to predispose the peritoneal surface to tumor cell implantation,\textsuperscript{108,109} and due to the incidence of port site metastasis, laparoscopic procedures for malignant conditions are controversial.\textsuperscript{110,111}

\subsection*{1.6.2 - Local peritoneal acidosis}

Local peritoneal acidosis is known to occur in laparoscopy, however it is unclear if this is due to hypoxia of the tissues due to increased IAP or associated with local absorption of CO$_2$.\textsuperscript{99} This local acidosis and irritation is thought to contribute to peritoneal tissue damage and morphological changes as described above.\textsuperscript{60,112} Several \textit{in vitro} and research studies have compared intracellular pH with a pneumoperitoneum created with CO$_2$ and alternative gases such as nitrous oxide and helium.\textsuperscript{99,113} Wong et al. (2005) found in pigs that ambient gas environment was a determinant of parietal peritoneal pH and while intra-abdominal pressure contributed to local acidosis it was of minor significance.\textsuperscript{99}

Peritoneal acidosis has been shown to offer some immune-protection.\textsuperscript{22,101} Hanley et al. (2007) documented decreased levels of the pro-inflammatory cytokine TNF-\(\alpha\) and increased serum levels of anti-inflammatory cytokine IL-10 in rats exposed to STCO$_2$ insufflation.\textsuperscript{101} This immunomodulation is thought to be due to acidification of peritoneal macrophages, however the clinical importance of this is unknown.\textsuperscript{101}

\subsection*{1.6.3 - Adhesion Formation}

Surgical stimulation and the sequential inflammatory response can lead to the formation of adhesions if vascular injury occurs.\textsuperscript{98} The propensity for adhesion formation is species dependent and is a significant contributor to morbidity in human patients.\textsuperscript{21,98} While it has been shown by some authors that laparoscopy reduces adhesion formation, compared to laparotomy, it does not completely eliminate them,\textsuperscript{114–116} and the impact of CO$_2$ insufflation on adhesion formation is inconclusive.\textsuperscript{117,118}
1.7 - Pain following laparoscopy

Pain is an unpleasant sensory and emotional experience, associated with actual or potential tissue damage or described in terms of such damage.\textsuperscript{119} Although all animals experience pain, expression of pain varies with age, species and between individuals.\textsuperscript{120} Physiologic and behavioral responses can be evaluated and used to score the severity of pain in order to quantify it.\textsuperscript{121} Postoperative pain following a laparoscopic procedure while less severe than for a laparotomy, is still a source of surgical stress.\textsuperscript{122} A major component of postoperative pain in laparoscopy is associated with abdominal distension and CO\textsubscript{2} insufflation, due to irritation and stretching of the phrenic nerves causing referred shoulder pain.\textsuperscript{14,123,124}

1.7.1 - Assessment of Pain

Currently no fully validated subjective instruments are available for assessment of pain in dogs and cats.\textsuperscript{125} There are a variety of pain scales reported in veterinary literature including the visual analogue scale, Melbourne pain scale, Colorado acute pain score, and the Glasgow composite pain scale.\textsuperscript{126–128}

The visual analogue scale commonly used in human medicine has been found to be insensitive in veterinary patients.\textsuperscript{125,128} As such there has been development of multiple pain scales from tertiary referral centers throughout the world. These scales vary in their content, scale, and ease of use. The Melbourne pain scale incorporates physiologic variables such as blood pressure and appetite as well as psychomotor changes, protection of the wound and vocalization.\textsuperscript{127} The Colorado acute pain scoring system is based on behavior and response to palpation with pictorial guidelines to help practitioners.\textsuperscript{126} The Glasgow composite measure pain scale is behavior based and designed to be quick, easy, and applicable to a variety of breeds undergoing a variety of procedures.\textsuperscript{121,129,130}

The Glasgow composite measure pain scale has been validated in dogs and is commonly used in clinical practice.\textsuperscript{36,121,129,131} The Glasgow composite measure pain score has been used to evaluate the analgesic properties of tramadol, hydrocodone, and
ice compression following a tibial plateau leveling osteotomy in client owned dogs.\textsuperscript{132,133} It has also been used to compare postoperative discomfort in dogs undergoing lift laparoscopy compared to STCO\textsubscript{2} insufflation.\textsuperscript{134}

1.8 - Altered pneumoperitoneum environments

The proposed thermoregulatory benefits of altering the temperature and humidify of insufflated CO\textsubscript{2} has been evaluated extensively for laparoscopic surgery in humans.\textsuperscript{58,135–139} Multiple devices have been developed in order to warm and humidify the insufflated gas, however the Insuflow device manufactured from Lexion Medical is the most widely studied product.\textsuperscript{38,58,135,140–142} Alternative devices have been evaluated that only warm or humidify the insufflated CO\textsubscript{2}, such devices include the Thermoflater from Karl Stroz,\textsuperscript{143,144} which warms CO\textsubscript{2} with a heated coil wrapped around the insufflation tubing, and the Aeroneb by Philips which saturates the gas particles with infiltration of sterile saline.\textsuperscript{30}

1.8.1 - Warmed (W) CO\textsubscript{2} insufflation

The theory for warming insufflated CO\textsubscript{2} was postulated as traditional STCO\textsubscript{2} is 15°C cooler than canine core body temperature,\textsuperscript{47} with a decrease in esophageal temperature of 0.3°C for every 50 L of STCO\textsubscript{2} insufflated.\textsuperscript{29} It was suggested warming this insufflated gas may help prevent or minimize perioperative hypothermia. Several studies have failed to show significant benefit to this and many have actually shown detrimental effects associated with an increased pain response.\textsuperscript{143,145,146}

Nelskyla et al. (1999) used warmed CO\textsubscript{2} insufflation in 37 women undergoing laparoscopic hysterectomy and found warming of the insufflated gas offered no protection against hypothermia, with the warmed group actually having a greater decline in core body temperature compared to the control group.\textsuperscript{147} In contrast Backland et al. (1998) found a protective thermoregulatory effect of warmed CO\textsubscript{2} insufflation in people, however the volume of CO\textsubscript{2} insufflated was significantly greater in the STCO\textsubscript{2} group, which may have contributed more to evaporative heat loss.\textsuperscript{148} This study also found a significantly greater cardiac index and urine output in the warmed group, suggesting the
use of this altered pneumoperitoneum environment may be of benefit in patients with renal disease.\textsuperscript{148}

\subsection*{1.8.2 - Humidified (H) CO$_2$ insufflation}

The saturation of gas particles not only prevents evaporative heat losses, it also preserves the peritoneal surface from desiccation, reducing adhesion formation.\textsuperscript{59,149,150} Humidification of ambient room temperature gas requires less water for saturation than that of warmed gas (18 mg at 21°C compared to 44 mg at 37°C) and makes it possible to reduce evaporative heat loss by 50%.\textsuperscript{151} Humidification alone however has had only moderate success and while one clinical human trial and research porcine studies have found a protective thermoregulatory effect,\textsuperscript{30,151,152} when compared to the warming and humidification of insufflated CO$_2$, the combination has been found to be the most effective.\textsuperscript{30,151}

\subsection*{1.8.3 - Warmed and Humidified (WH) CO$_2$ insufflation}

Warmed and humidified CO$_2$ insufflation has been reported to prevent perioperative hypothermia, reduce peritoneal irritation, adhesions, and patient nausea, while improving patient comfort, hemodynamic data, urine output, and visibility for the surgeon by minimizing lens fogging.\textsuperscript{58,135–139}

\subsection*{1.8.3.1 - In vitro studies}

In 2002, Johnston et al. evaluated peritoneal cell desiccation and viability following exposure to STCO$_2$ and WHCO$_2$.\textsuperscript{153} This was measured by incorporation of fluorescent dye into the DNA of dead or dying cells. Significant cell DNA fragmentation and cell death occurred in the STCO$_2$, however cell viability was maintained with WHCO$_2$.\textsuperscript{153}
1.8.3.2 - Experimental animal studies

Multiple rodent and porcine studies have been performed to evaluate the thermoregulatory effect, with many finding a benefit.\textsuperscript{104,138,154–156} Noll et al. (2012) exposed four pigs to four differing CO\textsubscript{2} environments with 8-day intervals between each procedure. The treatment groups included no pneumoperitoneum (control), STCO\textsubscript{2}, HCO\textsubscript{2} and WHCO\textsubscript{2}. Heat loss was recorded at 4 hours after 720 L CO\textsubscript{2} had been insufflated. Heat loss for the STCO\textsubscript{2} was 3.63°C ± 0.31 compared to 1.98°C ± 0.09 in WHCO\textsubscript{2} (\(P=0.0024\)), with the temperature decrease becoming statistically significant between the groups after 50 minutes (\(P=0.04\)). The authors concluded that WHCO\textsubscript{2} was of benefit if the procedure was over 60 minutes in duration.\textsuperscript{138}

In addition to a reduction in heat loss it has been postulated that the desiccation and damage of peritoneal cells seen in STCO\textsubscript{2} insufflation is avoided with the use of WHCO\textsubscript{2}.\textsuperscript{117,157,158} Glew et al. (2004) reported faster dissipation of insufflated CO\textsubscript{2} in piglets preserved the peritoneal surface, resulting in less inflammation and less postoperative pain.\textsuperscript{154} Several rodent studies have validated this theory finding preservation of intercellular clefts, mesothelial cells and microvilli when treated with WHCO\textsubscript{2}.\textsuperscript{20,102,103} Davey et al. (2013) evaluated the peritoneal surface following exposure to STCO\textsubscript{2}, WCO\textsubscript{2} and WHCO\textsubscript{2} for a 2-hour period. Twelve hours following recovery peritoneal tissue samples were harvested and analyzed with scanning electron microscopy.\textsuperscript{103} Rats exposed to STCO\textsubscript{2} and WCO\textsubscript{2} insufflation had rounding of mesothelial cells, crenation and collapse of microvilli; however those exposed to WHCO\textsubscript{2} had closely apposed mesothelial cells with prominent microvilli and appeared similarly to the control group in which no insufflation was performed.\textsuperscript{103}

1.8.3.3 - Randomized clinical human trials

The largest amount of literature on the subject of WHCO\textsubscript{2} insufflation in laparoscopy comes from randomized clinical trials in the human medical field with key variables of core body temperature, and postoperative pain.\textsuperscript{38,58,135–137,139,159–166}
Table 1.2 - Randomized human clinical trials reporting the core body temperature and pain in WHCO\textsubscript{2} compared to STCO\textsubscript{2} insufflation.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Temperature</th>
<th>Pain</th>
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<td>Ott, Reich, Love et al. 1998</td>
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<td>Mouton, Bessell, Otten et al. 1999</td>
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<td>Champion &amp; Williams 2006</td>
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<td>Davis, Mikami, Newlin et al. 2006</td>
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<td>Manwaring, Readman, Maher 2008</td>
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In a randomized double-blinded prospective clinical trail of 30 patients undergoing laparoscopic Roux-en-Y gastric bypass, core body temperature was found to be higher in those treated with WHCO\textsubscript{2} (36.2°C ± 5 compared to 35.7°C ± 6 in STCO\textsubscript{2}) ($P = 0.02$), however pain was not statistically different and the authors concluded the use of WHCO\textsubscript{2} was of limited clinical benefit.\textsuperscript{159} Klugsberger et al. (2014) demonstrated a reduction in pain 6 hours postoperatively following a laparoscopic cholecystectomy but as with the above mentioned study while a temperature difference (WHCO\textsubscript{2}; 37.07°C ± 0.35, STCO\textsubscript{2}; 36.8°C ±0.46 ($P = 0.01$)) was found between treatment groups the results were considered of limited clinical benefit.\textsuperscript{160}

In a prospective randomized trial patients undergoing laparoscopic cholecystectomy with STCO\textsubscript{2} or WHCO\textsubscript{2}, it was found that the intraperitoneal cytokine response was reduced in patients receiving WHCO\textsubscript{2} insufflation.\textsuperscript{167} In a 2002 study by Ott, 52 patients undergoing laparoscopy for benign reasons had a pneumoperitoneum established with STCO\textsubscript{2} or WHCO\textsubscript{2} using the Insuflow device.\textsuperscript{168} IL-6 and CRP serum samples were obtained every 6 hours for 72 hours and a significant difference between the treatment groups was reported.\textsuperscript{168} A decrease in plasma IL-6 concentration from 24 pg/mL to 6 pg/mL and a decrease in CRP concentration from 23 mg/dL to 5 mg/dL was
noted between the STCO₂ and WHCO₂ treatment groups.¹⁶⁸ It was concluded that the decreased inflammatory response seen with WHCO₂ was an indication of reduced peritoneal stress and trauma.¹⁶⁸ In contrast other studies have found no significant difference in CRP or IL-6 concentrations between the two treatment groups.¹⁶⁶,¹⁶⁹

A double blinded randomized control trial in 100 patients undergoing laparoscopic vaginal hysterectomies found a significant difference in the level of shoulder tip pain with lower pain scores reported in WHCO₂ compared to STCO₂ insufflation.¹⁶¹ This finding was also reported by Champion et al. (2006) with a reduction in shoulder pain seen at 18 hours.¹⁶⁶ The authors however, concluded that this finding was not clinically relevant and the additional cost, and technology of the Insuflow device was of no clinical benefit.¹⁶⁶

Results are conflicting with a significant volume of the literature published by authors with financial conflicts of interest.³⁸,⁵³,⁶⁸,¹⁷⁰–¹⁷² In a large meta-analysis conducted by the Cochrane Collaboration a mild benefit was seen in core body temperature with the use of WHCO₂ in comparison to STCO₂, however the authors felt this was not clinically significant with a difference of 0.3°C reported.¹⁷³ In addition no effect on postoperative pain, length of hospitalization, lens fogging, or length of operation, were seen.¹⁶³,¹⁷³ The authors state that while a small difference in core body temperature was seen the use of WHCO₂ did not account for improved patient outcomes, therefore concluded there was no clear evidence for the use of warmed compared to cold gas insufflation with or without humidification.¹⁷³

1.9 - Summary of Literature Review

In conclusion a literature review of STCO₂ insufflation used for laparoscopy in human and veterinary medicine identified several detrimental effects, including hypothermia, cardiorespiratory depression, acidosis, peritoneal damage and postoperative pain.²²,²⁴–²⁶,¹⁰⁷ Of particular interest due to its wide spread implications is the potential for exacerbation of perioperative hypothermia.²⁹ Hypothermia is thought to develop in STCO₂ due to evaporative heat lost in saturating insufflated CO₂ particles.¹⁵¹ Perioperative hypothermia increases the risk for prolonged recovery, coagulopathies, delayed wound healing, and organ dysfunction.⁴⁰,⁴¹ The cardiorespiratory considerations
during laparoscopy are also of importance. A drop in venous return due to the collapse of the inferior vena cava and vasodilation secondary to hypercapnia is a significant concern owing to the potential implications to cardiac output. In most studies it appears that while a reduction in stroke volume is evident cardiac output is preserved due to the cardiostimulatory effects of CO\textsubscript{2} and subsequent tachycardia. Additionally most cardiorespiratory alterations appear transient and while of consideration appear well compensated for in the healthy patient.

While laparoscopy demonstrates a reduced inflammatory and pain response compared to laparoscopy, efforts are being made to minimize these effects further. Widespread peritoneal cell damage is reported with STCO\textsubscript{2} which may predispose to adhesion formation or tumor cell implantation. Postoperative pain in laparoscopy is associated with the stretch or irritation of the phrenic nerve and referred shoulder pain.

In an effort to minimize these detrimental effects alternative pneumoperitoneum environments have been evaluated. WHCO\textsubscript{2} has been postulated to reduce perioperative hypothermia, reduce inflammation, peritoneal damage, and postoperative pain. There are conflicting results in the human and experimental animal research. In a Cochrane review no significant clinical benefit was found with the use of warmed CO\textsubscript{2} insufflation with or without humidification. It is currently unknown if the use of WHCO\textsubscript{2} would provide any benefit in veterinary patients, however due to variation between species peritoneal surface area and the volume of CO\textsubscript{2} used for insufflation the use of this modality warrants investigation.

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157. Ott D. Desertification of the Peritoneum by Thin-Film Evaporation During


CHAPTER II: Effect of warmed and humidified carbon dioxide for pneumoperitoneum on core body temperature, cardiorespiratory variables, thromboelastography, systemic inflammation, peritoneal response, and postoperative pain during laparoscopy in healthy mature dogs.

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2.1 - Abstract

Objective – To evaluate the effect of warmed, humidified carbon dioxide (WHCO₂) pneumoperitoneum on core body temperature, cardiorespiratory variables, thromboelastography, systemic inflammation, peritoneal response, and postoperative pain in healthy, mature, dogs undergoing laparoscopy.

Design – Randomized, crossover study.

Animals – Mature, purpose-bred dogs (n=6).

Procedures – Each dog, in two separate anesthetic episodes, had a pneumoperitoneum created using differing CO₂ environments; standard CO₂ (STCO₂: 22°C, 0% relative humidity), and WHCO₂ (37°C, 98% relative humidity). During each episode, data collected included core body temperature, cardiorespiratory parameters, thromboelastography, and inflammatory biomarkers. All dogs had peritoneal biopsies evaluated with scanning electron microscopy and were assessed for postoperative pain.

Results – Mean core body temperature was significantly lower (35.2 °C, 95% CI 34.5 – 35.8 °C) with WHCO₂ compared to STCO₂ (35.9 °C, 95% CI 35.3 – 36.6 °C) across all time points. Cardiac Index increased over the duration of the procedure for both treatments and was not significantly different between treatments. No significant difference in thromboelastography was found between groups as indicated by the coagulation index. Subjective evaluation of peritoneal biopsies revealed mesothelial cell loss with STCO₂. There was no significant difference in circulating C-reactive protein or IL-6. There was a significant increase in the number of postoperative pain scores above 0 in the WHCO₂ treatment group.

Conclusions and Clinical Relevance – These data suggest the use of WHCO₂ for pneumoperitoneum in healthy, mature, dogs undergoing laparoscopy did not differ from the use of STCO₂.

2.2 - Introduction

Laparoscopy involves insufflation of the abdomen using CO₂ to create a working space allowing for maneuvering of the endoscope and instruments.¹ Laparoscopic procedures in dogs reduces postoperative pain, surgical stress, and improves postoperative recovery compared to traditional open approaches.²⁻⁴ Because of these
benefits, laparoscopy is commonly performed nowadays in veterinary medicine.\textsuperscript{5,6}

Insufflation with STCO\textsubscript{2} for laparoscopy has a temperature of 22°C and 0% relative humidity.\textsuperscript{7–9} Canine core body temperature ranges from 37.5°C to 39.2°C,\textsuperscript{10} which is at least 15°C higher than that of the insufflated CO\textsubscript{2}. The use of STCO\textsubscript{2} insufflation is a significant cause of hypothermia in human patients undergoing laparoscopic procedures due to heat loss from evaporation of liquid from the peritoneal surface in order to saturate the insufflated CO\textsubscript{2}, at a rate of 0.3°C drop in core body temperature for every 50 L of CO\textsubscript{2} insufflated.\textsuperscript{9} The effect of using STCO\textsubscript{2} on core body temperature in dogs is presently unknown.

Perioperative hypothermia in dogs is defined as a body temperature < 36.5°C.\textsuperscript{11} This has detrimental effects leading to patient morbidity in different species, including cardiac, hepatic, and renal dysfunction, coagulopathy, increased transfusion requirements, impaired humoral and cellular immunity affecting wound healing, prolonged recovery, and altered drug metabolism.\textsuperscript{11,12} Inadvertent perianesthetic hypothermia is one of the most common complications with anesthesia,\textsuperscript{12} as 84% of ~1500 dogs undergoing general anesthesia for surgical or diagnostic procedures, were classified as hypothermic at the time of anesthetic recovery.\textsuperscript{11}

Studies investigating the hemostatic effects of hypothermia in humans show hypocoagulability in the clinical patient and impairment of hemostasis \textit{in vitro} as measured by TEG.\textsuperscript{13} Even mild perioperative hypothermia (decrease in core body temperature by 1-3°C) resulted in increased bleeding times during surgery, higher rates of blood loss, and increased need for blood transfusion in people undergoing surgery.\textsuperscript{14} Similar to humans, intraoperative hemorrhage may be exacerbated by perioperative hypothermia in dogs leading to complications such as need for blood transfusion, hypoxia, and even death.\textsuperscript{15}

Strategies to prevent perioperative hypothermia are required to ensure safe perioperative outcomes for surgical patients undergoing laparoscopy. Warming and humidification of CO\textsubscript{2} (37°C, 97% relative humidity) (WHCO\textsubscript{2}) for creation of pneumoperitoneum has been evaluated extensively for laparoscopic surgery in humans by investigating core body temperature, postoperative pain, recovery time, postoperative nausea, urine output, lens fogging, and hemodynamic data.\textsuperscript{16–21} In 50 morbidly obese
patients undergoing gastric by-pass surgery, WHCO₂ resulted in increased intra-operative core body temperature and reduced postoperative shivering compared to STCO₂. In contrast, additional adult human clinical trials have found no difference in intraoperative core body temperature between the CO₂ treatments. A study in 30-35 kg pigs comparing insufflation devices found that WHCO₂ used for pneumoperitoneum and the duration of the procedure impacted heat loss, with heat loss in WHCO₂ being significantly less than that in STCO₂ only after 50 minutes. In dogs, the higher surface area compared to humans may result in benefits of using WHCO₂ in providing thermoregulatory protection.

Apart from the potential thermoregulatory benefits, WHCO₂ creates a less irritant environment which results in reduced patient discomfort in humans. Experimental studies in rats have shown that WHCO₂ insufflation during laparoscopy reduces peritoneal injury and adhesion formation as seen with scanning electron microscopy. It is this preservation of peritoneal tissues from desiccation that is thought to mediate a reduced systemic inflammatory response as indicated by a reduction in the level of circulating C-reactive protein (CRP) and Interleukin-6 (IL-6). Both CRP and IL-6 are inflammatory mediators used as biomarkers to assess the presence and degree of tissue damage. These are sensitive indicators of surgical trauma and inflammation in dogs.

More advanced laparoscopic procedures are being performed in veterinary medicine which require prolonged periods of pneumoperitoneum (e.g. cholecystectomy, ureteronephrectomy, adrenalectomy), yet the effect of WHCO₂ in dogs undergoing laparoscopy has not previously been investigated. Evaluation of WHCO₂ is required in an attempt to optimize postoperative patient recovery and outcome.

The objective of this research was to evaluate the effect of WHCO₂ pneumoperitoneum on cardiorespiratory parameters, core body temperature, systemic inflammatory response, coagulation, peritoneal morphology and postoperative pain in healthy, mature, research dogs undergoing laparoscopy. Our hypothesis was that the use of WHCO₂ would result in better thermoregulation, less inflammatory response, better cardiovascular function, and less pain than STCO₂.
2.3 - Materials and Methods

2.3.1 - Animals

Six healthy, mature aged (> 6 years old) purpose-bred Beagles, weighing between 9.6-13.7 kg were used in this study. All procedures were performed in the comparative clinical research facility (CCRF), Ontario Veterinary College, University of Guelph and were in accordance with the University of Guelph Animal Utilization Protocol. All dogs were housed in the Central Animal Facilities, University of Guelph and cared for in compliance with the Canadian Council on Animal Care guidelines and the Animals for Research Act. An acclimatization period of 7 days was allowed prior to any surgical intervention.

Prior to entry into the study all dogs had a complete blood cell count, biochemical profile, and physical examination performed.

2.3.2 - Experimental design

Dogs were randomized in a cross-over study design. The CO$_2$ environment (STCO$_2$ [22°C, 0% relative humidity] or WHCO$_2$ [CO$_2$; 37°C, 98% relative humidity]) selected for each occasion was randomized using a random number generator, with at least a 2-week washout period between treatments.

2.3.3 - Anesthesia and instrumentation

Dogs were fasted for 12 hours in preparation for anesthesia. On the day of surgery, a 20-gauge, 1.88inch (4.78 cm) catheter was placed in the cephalic vein and each dog premedicated with hydromorphone (0.05 mg/kg, IV) prior to induction with propofol (2-4 mg/kg, IV). The dogs were intubated and anesthesia maintained with an end-tidal iso-flurane concentration (ET$_{EISO}$) of 1.6%, measured with an infrared gas analyzer, calibrated before each experiment with the recommended standardized calibration gas mixture. Dogs were also instrumented for electrocardiography, HR, CVP, direct arterial pressure (SAP, DAP, and MAP), esophageal temperature, ET$_{CO_2}$, and specific spirometry variables (tidal volume, respiratory rate, peak inspiratory pressure) using a multiparameter monitor. Intermittent positive pressure mechanical ventilation
was initiated to maintain an initial ET\(\text{CO}_2\) of 40 mm Hg using a volume-cycled ventilator.\(^h\) Subsequent end-tidal CO\(_2\) concentrations during the experiment were recorded without adjusting ventilator settings. Standard practices for maintaining core body temperature of dogs were performed, including use of a warming water blanket and warmed air.\(^i\) Room temperature and humidity was measured using a thermohygrometer\(^j\) at all time points. Intravascular volume support was provided throughout the procedure using an isotonic balance solution\(^k\) at 3-5 mL/kg/hr IV.

A central vein 18-gauge double lumen catheter\(^l\) was placed in the left jugular vein and a 20-gauge, 1-inch (2.54 cm) catheter in the dorsal pedal artery for collection of central venous and arterial blood samples for measurement of pH, gas tensions, and central venous and arterial oxygen saturation (\(\text{ScvO}_2\), \(\text{SaO}_2\)), lactate concentrations, electrolytes, and hemoglobin. Measurements of CO were determined by LiDCO\(^m\) by attaching a lithium chloride sensor\(^n\) to the side port of a three-way valve connected to the arterial catheter. Extension tubing was attached to the three-way valve, connected to a blood collection bag,\(^m\) and blood passed through a peristaltic pump that produced a blood flow of 4 mL/min across the sensor.\(^m\) The hemoglobin concentration and serum sodium concentration required by the LiDCO computer were determined by use of a blood gas analyzer,\(^o\) immediately prior to obtaining CO measurement. The dose of lithium chloride\(^p\) (0.006 mmol/kg) was injected into an extension set attached to the jugular vein catheter and flushed with 8 mL of saline (0.9% NaCl) eight seconds after starting the injection phase on the LiDCO computer.

Each dog was positioned in dorsal recumbency and a standard, three-step aseptic preparation of the ventral abdomen was performed. A 0.5 cm, subumbilical incision was made through the skin, subcutaneous tissues and linea alba, to allow placement of the camera portal using a standard 6 mm laparoscopic cannula\(^q\) with a Hasson technique. A purse-string suture was placed surrounding the cannula in order to minimize leakage of CO\(_2\). Standard insufflation tubing or specialized tubing connected to the Insuflow device\(^r\) designed to warm and humidify insufflated CO\(_2\), was attached to the mechanical insufflator.\(^s\) The Insuflow device heats CO\(_2\) via a heated coil attached to the insufflator.\(^s\) This warmed CO\(_2\) passes through the insufflation tubing towards the patient and is then humidified through a chamber of sterile water prior to entry into the abdomen.
Pneumoperitoneum was induced with CO₂ to an intra-abdominal pressure of 10 mmHg with a mechanical insufflator.⁶ A 0°, 5 mm x 29 cm laparoscope⁷ was inserted into the abdomen through the camera portal and a brief exploration of visible abdominal organs was performed. An instrument portal was created halfway between the subumbilical portal and the pubis using laparoscopic guidance, to allow placement of a 6 mm laparoscopic cannula.⁸

2.3.4 - Cardiorespiratory measurements

In each dog, cardiorespiratory parameters and core body temperature were measured at baseline, 5, 15, 30, 45, 60, and 90 minutes following initiation of CO₂ pneumoperitoneum and 5 minutes following desufflation. Measurements of CO, SAP, DAP, MAP, HR, CVP, SaO₂, ScvO₂, arterial and central venous blood gas tensions, electrolyte concentrations, and lactate concentrations, ET₅₀ and ET₇₅ concentrations, and spirometry variables. From these variables, cardiac index (CI), stroke volume (SV), stroke volume index (SVI), systemic vascular resistance (SVR), arterial and central venous oxygen content (CaO₂, CcvO₂), oxygen consumption (VO₂) and oxygen delivery (DO₂), and extraction oxygen ration (ERO₂) were calculated.⁷ ²⁷,²⁸ The dose of propofol required for induction, time from induction to baseline recording, time of surgery and anesthesia were recorded.

2.3.5 - Tissue and blood samples

Three-mL of citrated blood was collected for packed red blood cell volume, total protein, and global hemostasis testing using kaolin-activated TEG⁹ at baseline, immediately upon completion of the procedure, 24 and 48 hours following completion of the procedure. One-mL of citrated blood was pipetted into a kaolin vial⁹ and gently inverted 5 times. Thromboelastography was performed using 340 µL of kaolin activated blood pipetted into the cup with 20 µL of 0.2M CaCl₂ added. Measurements were performed at the standard machine channel temperature of 37°C. The reaction time (r) indicated the time taken (min) until a clot was first detected (normal range 2 – 7 min). The maximum amplitude (MA) in mm (normal range 47 – 68 mm) and clot elasticity (G)
in dynes/sec (normal range 4.5 – 8.5 dynes/sec) were used as measures of clot strength, indicating platelet function and fibrinogen levels. The coagulation index (CoI) was used as an overall assessment of coagulability, by taking into account the relative contribution of all the TEG measurements (normal range -3 – 3).\textsuperscript{29} The reference range for these are specific to the Ontario Veterinary College and are based on previous data. Blood samples were collected from the left jugular catheter into plain serum tubes to measure CRP and IL-6, at baseline, 1, 4, 12, 24 and 48 hours post initiation of pneumoperitoneum.

A peritoneal biopsy was collected at 5, 30 and 90 minutes following initiation of CO\textsubscript{2} pneumoperitoneum, using 5 mm laparoscopic cup biopsy forceps\textsuperscript{w}, introduced through the instrument port and submitted for scanning electron microscopy. The first biopsy was collected from the right lateral abdominal wall just caudal to the last rib, approximately 10 cm dorsal to ventral midline. Each subsequent biopsy was collected 2-5 cm caudal to the previous sample. In the second procedure the biopsies were collected from the left lateral abdominal wall in a similar fashion. The tissues were fixed in a 2.5 % glutaraldehyde in a 0.1 M sodium phosphate buffer solution (pH 7.3) for 1-2 weeks at room temperature. The samples were then washed in 0.1 M sodium phosphate buffer (pH 7.4) for 30 minutes before post-fixing in 1% osmium tetroxide, in a 0.1 M sodium phosphate buffer solution, for 2 hours. They were then dehydrated in an ascending ethanol series: 50%, 75%, 95%, 100% ethanol for 15 minutes, then dried with a critical point dryer. The samples were gold/palladium sputter-coated\textsuperscript{x} prior to visualization using a scanning electron microscope\textsuperscript{y} at an accelerating voltage of 10 kV. Electron micrographs were taken and reviewed by a boarded pathologist (RF). A subjective assessment of the degree of peritoneal desiccation and desquamation was graded. The authors (JS, RF) designed a scoring system where each variable were assigned a value between 0 and 4 as follows: 0= skeletal muscle present but no mesothelial cells, 1= serosal connective tissue present but no mesothelial cells, 2= serosal connective tissue present but few mesothelial cells, 3= serosal connective tissue present and intermediate number of mesothelial cells, 4= serosal connective tissue present and normal mesothelium.
2.3.6 - Recovery and pain score

At the end of the procedure all instruments were removed and pneumoperitoneum purged. The volume of CO\(_2\) used was recorded for each dog. The port sites were locally infiltrated with lidocaine (2 mg/kg)\(^z\) and the incisions closed routinely. The dogs were recovered from anesthesia, and anti-inflammatory medication provided (meloxicam; 0.1 mg/kg, IV).\(^{aa}\)

An investigator blinded (JK) to treatment group evaluated recovery from anesthesia and postoperative pain using the Short-Form Glasgow Composite Pain Score (CMPS-SF).\(^{30}\) All dogs were monitored continuously following anesthesia until standing, after which time they were assessed 4, 12, 24 and 48 hours postoperatively for any potential unanticipated pain. A score \(\geq 6\) was considered justification for rescue analgesia with hydromorphone (0.05 mg/kg IM). The dogs were returned to the research facility 48 hours post-procedure.

2.4 - Data analysis

Comparisons of core temperature, cardiorespiratory parameters, thromboelastography parameters, and inflammatory biomarkers between each treatment group in each dog were completed with a generalized linear mixed model with a random effect using a three factor-factorial design with repeated measurements over minutes for each dog; the three factors were treatment group, time (minutes) and sex.\(^{bb}\) Data was assessed for normality with a Shapiro-Wilk test. Backward step-wise regression was performed, Tukey and Dunnett’s corrections were made for pair-wised comparison with significance set at \(P \leq 0.05\). Normally distributed data were summarized as mean (95% CI). Data that were not normally distributed underwent logistic regression and were summarized as median (range) and odds ratio. Due to the scarcity of data in values greater than 0 for postoperative pain score this outcome was dichotomized to reflect either a score of 0 or above. Limited number of tissue samples were completely processed due to financial and timing constraints, therefore no meaningful statistical analysis was performed for peritoneal morphology seen at scanning electron microscopy.
2.5 - Results

There were 4 spayed female and 2 castrated male dogs with a median age of 7.5 years (range 6.4 – 10.3 years) and a median body weight of 11 kg (range 9.6 – 13.7 kg). There was no significant difference in body weight between the genders ($P = 0.095$). There was no difference between treatment groups in the dose of propofol used for induction ($P = 0.117$), $ET_{ISO}$ ($P = 0.189$), time from induction to baseline reading at 0 minutes ($P = 0.184$), surgery time ($P = 0.128$), or total anesthesia time ($P = 0.204$). (Table 2.1)

Table 2.1 - Anesthetic variables in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ (22°C, 0% relative humidity), or with WHCO$_2$ insufflation (37°C, 98% relative humidity). The values for all patients, over all treatment groups is reported as no significant difference in the dose of propofol required for induction, $ET_{ISO}$, time from induction to baseline reading at 0 minutes, surgery time or total anesthesia time. (Mean, 95% CI)

<table>
<thead>
<tr>
<th>Anesthetic variables</th>
<th>STCO$_2$</th>
<th>WHCO$_2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol (mg)</td>
<td>44 (30-58)</td>
<td>51 (38-66)</td>
<td>0.117</td>
</tr>
<tr>
<td>(Mean, 95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$ET_{ISO}$ (%)</td>
<td>1.75 (1.66-1.83)</td>
<td>1.71 (1.64-1.8)</td>
<td>0.189</td>
</tr>
<tr>
<td>(Median, range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from induction to baseline (min)</td>
<td>48 (37-58)</td>
<td>52 (42-63)</td>
<td>0.184</td>
</tr>
<tr>
<td>(Mean, 95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery time (min)</td>
<td>107(102-112)</td>
<td>111 (107-116)</td>
<td>0.128</td>
</tr>
<tr>
<td>(Mean, 95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anesthesia time (min)</td>
<td>173 (160-187)</td>
<td>181 (167-194)</td>
<td>0.204</td>
</tr>
<tr>
<td>(Mean, 95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean core body temperature was significantly lower (mean, 95% CI; 35.2 °C, 34.5 °C – 35.8 °C) in the WHCO$_2$ group compared to the STCO$_2$ group (35.9 °C, 35.3 °C – 36.6 °C) across all time points ($P < 0.0001$), despite no difference in room temperature ($P = 0.378$) or humidity ($P = 0.451$) between treatment groups. All dogs were classified
as hypothermic (< 36.5 °C) for the first 60 minutes of procedure; however the core body temperature increased in all patients after 30 minutes (P < 0.0001).

Heart rate (P = 0.656), CO (P = 0.273), CI (P = 0.873), SVI (P = 0.947), DO₂ (P = 0.790), ERO₂ (P = 0.240), CVP (P = 0.481), SVR (P = 0.619), CaO₂ (P = 0.167), CcvO₂ (P = 0.065), were no different between treatment groups; therefore, data are combined in Table 2.2. Heart rate increased over the duration of the procedure (P < 0.0001) (Figure 2.1).

![Heart Rate](image)

Figure 2.1 - Mean HR in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A sex interaction was noted over time (P = 0.027) with male patients having an elevation in HR earlier in the procedure than the female patients.

The CO and CI increased significantly from baseline following induction of pneumoperitoneum for the duration of the procedure (P < 0.0001) (Table 2.2) and a sex interaction was found on treatment group for CO (P = 0.023), but not for CI (P = 0.054). The P value for CO once adjusted was 0.161 (Figure 2.2).
Figure 2.2 - Median CO in healthy, mature, dogs undergoing laparoscopy with STCO2 and with WHCO2 insufflation. A sex interaction was found on treatment group for CO, with male patients having a significantly higher median CO with STCO2 insufflation compared to WHCO2 insufflation ($P = 0.023$). This was not observed for CI ($P = 0.054$) and the $P$ value for CO once adjusted was 0.161.

The SVI increased significantly from baseline following induction of pneumoperitoneum in the first 30 minutes of the procedure ($P = 0.003$) (Table 2.2). The mean CVP increased from baseline (9 mm Hg, 6 – 12 mmHg) following induction of pneumoperitoneum (13 mmHg, 10 – 17 mmHg) ($P < 0.0001$) and remained elevated for the first 30 minutes of the procedure. A sex interaction on treatment group was observed ($P < 0.0001$) with a higher CVP recorded in male patients treated with STCO2 (12 mmHg, 8 – 17 mm Hg) compared to WHCO2 (9 mm Hg, 5 – 14 mm Hg). Inversely a lower CVP was recorded in female patients treated with STCO2 (9 mm Hg, 6 – 13 mm Hg) compared to WHCO2 (12 mm Hg, 8 – 15 mm Hg). The SVR was significantly different over time ($P = 0.009$) (Figure 2.3), increasing following the induction of pneumoperitoneum and returning to baseline by the end of the procedure. A sex interaction on treatment group was also observed ($P = 0.047$) (Figure 2.4), however significance was not found with individual pair wise comparisons.
Figure 2.3 - Mean SVR in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A significant difference in mean SVR was found over time ($P = 0.009$), however no trends could be identified over time and significant confidence interval overlap is noted.

Figure 2.4 - Mean SVR in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A statistically significant sex interaction on treatment group was observed ($P = 0.047$) with a lower mean SVR recorded in male patients treated with STCO₂ compared to females.
Both CaO$_2$ ($P = 0.032$) and CcvO$_2$ ($P = 0.007$) decreased significantly over the duration of the procedure in both treatment groups. However, DO$_2$, VO$_2$ and ERO$_2$ increased following induction of pneumoperitoneum and remained elevated for the duration of the procedure ($P < 0.0001$) for both treatment groups (Table 2.2). Dogs in the WHCO$_2$ group had a significantly lower median VO$_2$ rate (2.4 mL/min, range 1.9 – 3.1 mL/min) compared to dogs in the STCO$_2$ group (2.8 mL/min, range 2.2 – 3.5 mL/min) across all time points ($P = 0.040$) (Table 2.3).

Table 2.2 - Cardiovascular parameters in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ (22°C, 0% relative humidity), or with WHCO$_2$ insufflation (37°C, 98% relative humidity). The values for all patients, over all treatment groups is reported as no significant difference was observed in HR, CI, SVI DO$_2$ or O$_2$ER between treatment groups.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Heart Rate (beat/min)</th>
<th>Cardiac Index (mL/min/kg)</th>
<th>Stroke Volume Index (mL/beat/kg)</th>
<th>Oxygen Delivery (mL/min)</th>
<th>Oxygen Extraction Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean, 95% CI)</td>
<td>(Median, range)</td>
<td>(Mean, 95% CI)</td>
<td>(Median, range)</td>
<td>(Mean, 95% CI)</td>
</tr>
<tr>
<td>0</td>
<td>104 (84-125)</td>
<td>112 (92-137)</td>
<td>1.1 (1.0-1.3)</td>
<td>22.3 (17.5-28.5)</td>
<td>6.2 (3.3-9.1)</td>
</tr>
<tr>
<td>5</td>
<td>114 (97-131)</td>
<td>152 (125-185) *</td>
<td>1.4 (1.2-1.6) *</td>
<td>30.6 (24.0-39.1) *</td>
<td>10.7 (7.8-13.6) *</td>
</tr>
<tr>
<td>30</td>
<td>122 (106-138)</td>
<td>165 (135-201) *</td>
<td>1.4 (1.3-1.6)</td>
<td>31.4 (24.6-40.1) *</td>
<td>10.1 (7.3-13.0) *</td>
</tr>
<tr>
<td>60</td>
<td>138 (122-154) *</td>
<td>159 (131-194) *</td>
<td>1.2 (1.0-1.4)</td>
<td>29.9 (23.4-38.2) *</td>
<td>12.7 (9.8-15.6) *</td>
</tr>
<tr>
<td>90</td>
<td>145 (128-161) *</td>
<td>168 (138-205) *</td>
<td>1.2 (1.0-1.4)</td>
<td>31.5 (24.7-40.3) *</td>
<td>10.4 (7.7-13.4) *</td>
</tr>
</tbody>
</table>

* Significant difference from Baseline (Time 0)
Table 2.3 - Median and range VO$_2$ in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ (22°C, 0% relative humidity) or with WHCO$_2$ insufflation (37°C, 98% relative humidity).

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Oxygen Consumption (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STCO$_2$</td>
</tr>
<tr>
<td>0</td>
<td>1.36 (1.09-1.7)</td>
</tr>
<tr>
<td>5</td>
<td>3.06 (2.45-3.81) *</td>
</tr>
<tr>
<td>30</td>
<td>3.25 (2.61-4.05) *</td>
</tr>
<tr>
<td>60</td>
<td>3.8 (3.05-4.73) *</td>
</tr>
<tr>
<td>90</td>
<td>3.29 (2.64-4.1) *</td>
</tr>
</tbody>
</table>

* Significant difference from Baseline (Time 0)
† Significant difference from STCO$_2$

Mean SAP, DAP, and MAP increased significantly from baseline following the induction of pneumoperitoneum ($P < 0.0001$). The mean MAP ($P = 0.006$) and DAP ($P = 0.040$) were significantly higher across all time points with STCO$_2$ (Figure 2.5)

![Mean Arterial Blood Pressure](image)
Figure 2.5 - Mean MAP in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. The mean MAP was significantly higher across all time points with STCO₂ compared to WHCO₂ insufflation ($P = 0.006$). A sex interaction was noted across time for the MAP ($P = 0.029$) and DAP ($P = 0.039$) with a more persistent arterial blood pressure elevation above baseline noted in female patients when pneumoperitoneum duration was greater than 15 minutes.

No significant difference in tidal volume ($P = 0.745$), end-tidal CO₂ ($P = 0.761$) or total volume of CO₂ used for insufflation ($P = 0.619$) was reported between treatment groups. ETCO₂ increased steadily over the duration of the procedure ($P < 0.0001$), however mean PaCO₂ initially dropped after induction of pneumoperitoneum (56 mm Hg, 52–60 mmHg to 49 mmHg, 46–53 mmHg), returning to baseline after 15 minutes ($P = 0.003$), and this change was not different between treatment groups ($P = 0.468$). A sex interaction was also noted ($P < 0.0001$) (Figure 2.6). A significant initial decline in PaCO₂ was seen in female patients in the first 5 minutes ($P < 0.0001$) followed by a steady increase, while male patients had limited variation throughout the procedure with PaCO₂.
Figure 2.6 - Mean PaCO₂ in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A sex interaction was observed over time with a significant decline in PaCO₂ within the first 5 minutes of the procedure in female patients (P < 0.0001).

No individual treatment effect was seen in mean venous pH (P = 0.369), however a gradual decline in venous pH was observed over time following an initial spike at the induction of pneumoperitoneum (P < 0.0001). Similar trends were found in mean arterial pH (Figure 2.7).

![Arterial pH](image-url)

Figure 2.7 - Mean pH in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. No clinically significant trends were found in mean arterial pH with an initial increase following induction of pneumoperitoneum (P < 0.0001), followed by a gradual decline over the remainder of the procedure. A sex (P < 0.0001) and treatment (P = 0.012) interaction was found over time, however no individual treatment effect was seen (P = 0.867).

Mean venous lactate increased over the duration of the pneumoperitoneum (P = 0.003) (1.64 mmol/L, 1.33 – 1.94 mmol/L to 2.53 mmol/L, 2.22 – 2.83 mmol/L) but no
A treatment effect was found \( (P = 0.067) \). Mean arterial lactate followed a similar trend (Figure 2.8).

![Graph of Arterial Lactate](image)

**Figure 2.8** - Mean arterial lactate in healthy, mature, dogs undergoing laparoscopy with STCO\(_2\) and with WHCO\(_2\) insufflation. Mean arterial lactate increased over the duration of the pneumoperitoneum \( (P = 0.008) \) with a treatment effect \( (P = 0.04) \) evident. A higher mean arterial lactate in STCO\(_2\) compared to WHCO\(_2\) across all time points. A treatment interaction also noted over time \( (P = 0.029) \).

There was no significant difference in any of the TEG measurements between treatment groups. There was no significant difference in the reaction time (r time) to form a clot between treatment groups \( (P = 0.330) \) or over time \( (P = 0.680) \). A sex effect was noted for r time \( (P = 0.023) \) (Figure 2.9). No significant treatment \( (P = 0.256) \) or sex effect \( (P = 0.089) \) was found for clot strength or maximum amplitude (MA), but it increased in the postoperative period \( (P < 0.0001) \) with a median MA of 72.4 mm (range 69.3 – 75.7 mm) 48 hours postoperatively, compared to a median MA of 63.9 mm (range 61.2 – 66.8 mm) at the end of the procedure. Similarly to MA, no significant treatment effect \( (P = 0.280) \) was found for clot elasticity (G), but an increase was noted in the postoperative period \( (P < 0.0001) \) with a median G of 13.3 dynes/sec (range 11.6 – 15.2
dynes/sec) 48 hours postoperatively, compared to a median G of 9.9 dynes/sec (range 8.7 – 11.4 dynes/sec) at the end of the procedure. No individual significant difference was noted in the overall coagulation index (CoI) between treatment groups ($P = 0.623$) but a sex effect ($P = 0.013$) (Figure 2.9) and event effect ($P = 0.001$) (Figure 2.10) were noted.

A - Male

Figure 2.9 - TEG trace in a healthy, mature, dog undergoing laparoscopy with STCO$_2$ insufflation 24 hours postoperatively. Sex effects were seen with r time ($P = 0.023$) and CoI ($P = 0.013$) with male patients having a tendency towards hypercoagulability (A - Male, B - Female).
Figure 2.10 - Mean Coagulation index (CoI) in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ and with WHCO$_2$ insufflation. An event effect was seen ($P = 0.001$) with CoI increasing in the postoperative period. A three-way interaction was found between treatment, time and sex ($P = 0.024$).

C-reactive protein was found to have a significant elevation in the postoperative period ($P < 0.0001$) (Figure 2.11). No significant difference was found between treatment groups ($P = 0.671$) or sex ($P = 0.207$). Due to the detectable limit of the Interleukon-6 (IL-6) assay (1 pg/mL) limited readable results were collected from the female patients. No treatment ($P = 0.561$) or event effect ($P = 0.571$) was found in the male patients who had a mean IL-6 of 6422 pg/mL (95% CI: 4603 – 8241 pg/mL).
Figure 2.11 - C-reactive protein (CRP) concentrations in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. CRP was found to have a significant elevation in the postoperative period compared to intra-operative measurements at 0 and 60 minutes ($P < 0.0001$).

Scanning electron microscopy did reveal some subjective differences between patients undergoing STCO₂ insufflation compared to those with WHCO₂ insufflation. WHCO₂ appeared to preserve the microvilli of mesothelial cells and the mesothelial cell layer (Figure 2.12) compared to STCO2 in which cell loss and desiccation occurred (Figure 2.13).
Figure 2.12 - Scanning Electron Microscopy (SEM) image at 10um of peritoneal biopsies taken at 90 minutes in a healthy, mature, dog undergoing laparoscopy with WHCO$_2$ insufflation. The WHCO$_2$ appeared to preserve the microvilli and the mesothelial cell layer.
Figure 2.13 - Scanning Electron Microscopy (SEM, 10um) image of peritoneal biopsies taken at 90 minutes in a healthy, mature, dog undergoing laparoscopy with STCO₂ insufflation. STCO₂ appeared to result in loss of microvilli of mesothelial cells, and mesothelial cell loss.

There was a significant increase in the number of postoperative pain scores above 0 in the WHCO₂ treatment group ($P = 0.041$) and patients were 13.9 times more likely to score above 0 if pneumoperitoneum was established with WHCO₂ compared to STCO₂; however, no patient received a score necessitating rescue analgesia. Pain scores above 0
decreased over time ($P = 0.012$) with patients more likely to have a score above zero in the first 4 hours postoperatively.

2.6 - Discussion

Results from this study suggest limited benefit for WHCO$_2$ for pneumoperitoneum in this cohort of six healthy, mature, purpose-bred dogs undergoing laparoscopy. Limited differences were recorded between treatment groups and the differences that were observed were of minor clinical significance. Additional studies are required to further evaluate the potential peritoneal mesothelium preservation prior to implementation of WHCO$_2$ in clinical cases.

Postoperative hypothermia has been shown to affect recovery time from anesthesia in both dogs and people. Sixty-nine dogs evaluated for the effects of hypothermia on recovery from general anesthesia had prolonged recovery times when hypothermic (< 37°C). Those dogs with a core body temperature of 35 - 35.4°C took 23.4 ± 22.1 minutes, until they were able to successfully rise to sternal recumbency, compared to normothermic (> 38°C) dogs who took 7.7 ± 3.8 minutes. In a human study, hypothermic patients scored for “fitness for discharge” were found to require 40 minutes longer to recover when body temperature was lower by 2°C, nearly doubling the duration of recovery.

Core body temperature in our study was found to be 0.7°C cooler in the WHCO$_2$ treatment group compared to STCO$_2$ across all time points. This counterintuitive temperature difference suggests a lack of protection of surgical hypothermia with the warming and humidification of CO$_2$ insufflation in this small cohort of dogs. The reason for the negligible protective effect is speculated to be due to the varying inter-species total peritoneal surface area, and the total CO$_2$ volume insufflated. The total peritoneal surface area (TPSA) in dogs compared to humans is unknown, but if the TPSA is significantly less this may contribute to less protection seen via warming of this surface. Another reason the expected thermoregulatory benefits were not observed in our study may be owing to radiation being the primary mechanism of heat loss in anesthetized patients, which is not addressed with WHCO$_2$. Multiple, randomized human clinical trials have found no difference in core body temperature between WHCO$_2$ and
STCO_{2}.^{18,19,21} In a recent meta-analysis, no changes in core body temperature were associated with warmed CO_{2} compared to standard CO_{2} with or without humidification.^{35} Farley et al. reported a questionable thermoregulatory benefit with a 0.3°C increase in intra-operative core body temperature in human patients undergoing laparoscopic cholecystectomy with WHCO_{2} compared to STCO_{2}.^{16}

In our study all dogs had standard measures for preservation of core body temperature. Warmed air blankets have been proven to minimize heat loss in the second phase of surgical hypothermia following redistribution^{15} and have been shown to increase core body temperature by 0.9°C compared to patients who received no active warming.^{36} Only one study evaluating the thermoregulatory benefits of WHCO_{2} used a heating blanket, in which no difference was found in core body temperature in human patients undergoing laparoscopic fundoplication.^{19}

The concern for CO_{2} associated heat loss during laparoscopy was first established by Ott et al. (1991),^{9} who postulated that hypothermia in laparoscopy was associated with liquid evaporation from the peritoneal surface to saturate the CO_{2} gas particles and related to the total volume of CO_{2} insufflated or to that leaking from port sites.^{7} Other researchers have found the relative contribution of CO_{2} heat loss to be minimal with the calculated amount of CO_{2} required to cause a drop by 1°C in a 70 kg person being 4800 L.^{37} The mean total volume of CO_{2} insufflated in our patients was 13 L, which was not significantly different between the treatment groups, but is a considerably smaller volume of CO_{2} compared to what is used in human laparoscopy.^{38} This minimal volume of CO_{2} used in the dogs of our study may help explain why no difference was seen in core body temperature with WHCO_{2}. Cases in which a higher volume of CO_{2} used may have found a stronger protective effect with the use of WHCO_{2} insufflation. Furthermore, in the dogs in our study, only one instrument port was placed and a minimal number of instrument passes were performed during peritoneal biopsy. This does not represent the clinical case in which a much larger number of instrument passes would occur allowing for CO_{2} leak and, subsequently, a greater volume of CO_{2} used. Regardless, it is unlikely additional instrument ports or more instrument passes would have considerably increased the total volume of CO_{2} used.
No clinically significant difference was noted between treatment groups in any of the cardiorespiratory parameters measured. Abdominal insufflation at high intra-abdominal pressures (> 15 mmHg) has been associated with reduced venous return and a subsequent reduction in CO.39–41 In two studies evaluating the effects of CO₂ insufflation in dogs undergoing laparoscopy a drop in stroke volume was noted but a concurrent increase in HR meant the CO was minimally affected.42,43 Cardiac output is the product of HR and SV; in this study, HR increased over the duration of the procedure and SVI increased for 30 minutes following induction of pneumoperitoneum. Consequently, both CO and CI increased and remained elevated throughout the procedure following the induction of pneumoperitoneum in both treatment groups.

In the study reported here pneumoperitoneum was induced with CO₂ to an intra-abdominal pressure of 10 mmHg similar to the clinical scenario. The paradoxical increase in CO in this study maybe associated with an increase in sympathetic flow due to abdominal wall stretching, surgical stimulation, and displacement of splanchnic volume from the increased abdominal pressure.40,42 A porcine study found a similar increase in CO, however the authors attributed this finding to an increase of individual pig HR and did not feel this was representative of the whole group.44

A treatment sex interaction was seen with males having a higher median CO with STCO₂ insufflation compared with WHCO₂ insufflation. Once a Tukey adjustment was performed this was not found to be significant, additionally the recorded difference in CO was 0.4 L/min, which is of questionable clinical significance as all values were considered within the acceptable reference range.28 This sex interaction may have been the result of an elevation in HR observed earlier in the procedure in males than females. In an upright human exercise study, females had lower CI than males, associated with an inability to adequately increase HR and accommodate for any drop in SV. This inability is postulated to be due to a blunted sympathetic response and higher vasodilatory state.45 In contrast other studies have found similar cardiorespiratory parameters during exercise in males and females.46,47

An increase in HR following induction of pneumoperitoneum in dogs was attributed an increase in sympathetic flow due to increased intra-abdominal pressure and nociception.42 This elevation could also occur with the surgical stimulation of port
placement and peritoneal biopsies in our study. Peritoneal biopsies were, however, limited to 5, 30 and 90 minutes and no spikes in heart rate were recorded at these times.

While an initial increase was observed in SVI this declined after 30 minutes, which is similar to reports from Shih et al. who found cats undergoing laparoscopic ovariohysterectomy had a drop in stroke volume for laparoscopic procedures (ovariectomy) over 30 minutes in duration.\(^4^3\) Likewise, a study in dogs also showed a decrease in SV from the onset of pneumoperitoneum with a decline in mean SV from 31.5 mL/beat to 25.5 mL/beat in the first 15 minutes of the procedure.\(^4^2\)

An increase in CVP was detected in the first 30 minutes of the procedure, indicating an increase in fluid volume and therefore an increase in right atrial pressure. Preload and its effect on SV and CO can be assessed through CVP, used as a surrogate measure and an indicator of volemia.\(^4^8\) No discernible trends in CVP have been previously reported by other studies evaluating cardiovascular parameters in dogs undergoing laparoscopy.\(^4^1,4^2\) Our research dogs were healthy and normovolemic, and the increase in CVP may be an artifact associated with transmission of an increased intra-abdominal pressure across the diaphragm.\(^4^4\)

Afterload, another factor of SV, is primarily determined by SVR, with vasodilation decreasing the amount of systolic wall stress the ventricle needs to overcome to eject volume. A reduction in SVR and afterload results in an increase in venous return and CO.\(^4^8\) No discernible trend was evident for SVR; however, a sex treatment interaction was noted with an elevated SVR in male patients treated with WHCO\(_2\) and inversely an elevation in female patients treated with STCO\(_2\). This is in concurrence with our other finding of an elevated CO in males treated with STCO\(_2\). The cause for this sex effect is unknown and may be due to the humidified CO\(_2\) having a slower evaporation rate in male patients with less subsequent hypercapnia and vasodilation. This partially fits with the findings of PaCO\(_2\) in male patients having limited variation over time.

The MAP and DAP were 5 mmHg higher in STCO\(_2\) compared to WHCO\(_2\) insufflation with females tending to have the most persistent elevations. While this finding was statistically significant, it is of limited clinical significance for the magnitude of change, and is probably related to an increase in SVR.\(^4^2\)
Abdominal insufflation with CO\textsubscript{2} in dogs can lead to hypercapnia and acidosis due to absorption in systemic circulation.\textsuperscript{49} Overall PaCO\textsubscript{2} declined after the induction of pneumoperitoneum returning to a baseline of 56 mm Hg after the first 5 minutes. In the face of an increasing ETCO\textsubscript{2} this result is due to alveolar dead space and ventilation/perfusion mismatch as indicated by the Bohr equation. Following PaCO\textsubscript{2} arterial and venous pH increased initially. This is in contrast to other studies in which arterial pH decreased in a more linear fashion over the duration of pneumoperitoneum.\textsuperscript{42,44,49,50} In the 2008 study by Duerr et al. peritoneal fluid pH was evaluated using non-sterile litmus paper, while other studies have validated peritoneal pH with the use of pH probes.\textsuperscript{49,51} Duerr et al. (2008) found that local peritoneal acidosis occurred within the first 20 minutes of insufflation and then appeared to stabilize, while systemic hypercapnia and acidosis persisted for the duration of the procedure. This lead to the conclusion the peritoneal acidosis was a local effect and not related to systemic absorption.\textsuperscript{49} While the use of non-sterile litmus paper precludes its use in a recovery study, peritoneal fluid pH can also be measured with a sterile pH probe,\textsuperscript{52,53} however the feasibility of this was beyond the scope of this study.

The increase observed in venous and arterial lactate concentration was attributed to reduced perfusion associated with an increase in intra-abdominal pressure (IAP) and anesthesia. In contrast Mayhew et al. (2013) found no difference in blood lactate over time or over a range of IAP 4-15 mm Hg in cats undergoing laparoscopy.\textsuperscript{50} The observation of an increased mean arterial lactate in STCO\textsubscript{2} compared to WHCO\textsubscript{2} and in females compared to males is likely clinically insignificant, as both values are considered within the normal reference range.\textsuperscript{54}

The partial pressure of arterial oxygen and consequently CaO\textsubscript{2} decreased over the duration of the procedure. This decrease was not physiologically significant with a minimum PaO\textsubscript{2} of 211 mm Hg. A drop in PaO\textsubscript{2} has been previously reported and is thought to be from ventilation perfusion mismatch.\textsuperscript{42} Muscle paralytics were suggested by the authors to help with any intra-pulmonary shunting by increasing thoracic compliance.\textsuperscript{42}

Values for DO\textsubscript{2}, VO\textsubscript{2}, and ERO\textsubscript{2} were all increased following induction of pneumoperitoneum and VO\textsubscript{2} was found to be higher in the STCO\textsubscript{2} group. The clinical
significance of this finding is limited with a median difference of 0.4 mL/min. The increase seen in our patient’s core body temperature over the duration of the procedure follows the same trend as oxygen consumption. We postulate that as the patients metabolic drive becomes higher with an increase in core body temperature, so too does their VO$_2$.

While the TEG variables MA, G and CoI significantly decreased in both treatment groups intra-operatively, the clinical significance of this finding is likely minimal as no values were below the normal reference interval at any time point. Coagulation impairments in hypothermic patients are well established and can be seen with core body temperatures under 37 °C, 12-14,32 however none of our patients would have qualified as being hypocoagulable. Conversely MA, G and CoI increased to above the established reference ranges suggesting a systemic inflammatory associated hypercoagulability in the postoperative period in both treatment groups. A general trend towards hypercoagulability was also evident in male dogs with the most significant difference seen at 24 hours postoperatively. This is in contrast to previous canine studies finding no sex effect on TEG results. 55 An inverse sex effect is reported in people in which females, especially pregnant females were found to be more coagulable than male patients due to increased concentrations of fibrinogen, factor VIII and decreases in Protein S. 56

No treatment effect was seen in CRP concentration but levels did increase postoperatively with the highest concentration recorded at 12 hours post operatively. This is in concurrence with previous reports of CRP concentration in dogs undergoing laparoscopic ovariohysterectomy. 26 The concentrations seen in our study are consistent with mild surgical inflammation with a mean of 32 mg/mL at 12 hours as the reported range for normal dogs is between 9-30mg/ml and upwards of 300 mg/mL for dogs with pyometra. 57

Unfortunately the detectable limit of the IL-6 assay was 1pg/mL and we found no females with a IL-6 concentration that was detectable. IL-6 is a cytokine that stimulates the production of CRP. No difference was found between the sexes for the serum concentration of CRP, therefore it is likely this result is due to a technical error. The canine IL-6 ELISA is not as well validated as the CRP ELISA but a range of 1-400
pg/mL has been reported in normal animals. In the male patients a mean of 6422 pg/mL was recorded which would be consistent with moderate surgical inflammation. The sex interaction noted is the inverse of that reported in human patients, in which females often have higher levels of inflammatory cytokines. The findings here are consistent with a higher sympathetic tone and subsequent suppression of pro-inflammatory cytokines, or more likely are a result of type I error.

In concurrence with other studies, a subjective assessment of peritoneal biopsies found evidence of mesothelial cell loss, as is associated with local peritoneal acidosis and mesothelial hypoxia in STCO2. The benefit of less peritoneal irritation in canine patients is currently unknown, and it is postulated that less peritoneal injury results in a lower likelihood of adhesion formation, a significant complication in human patients undergoing laparoscopic surgery. Peritoneal adhesions can lead to intestinal obstruction, chronic pelvic pain, female infertility, and difficulties at the time of reoperation. The denuded peritoneum as seen in STCO2 is also more susceptible to the implantation of cancer cells. Cai et al. have shown that WHCO2 reduced the cancer cell burden compared to STCO2 by inducing apoptosis, inhibiting proliferation, migration, invasion, and adhesion of human colon cancer cells. Port site metastasis is a common concern in human medicine, but has only been reported once in the veterinary literature. The importance of the peritoneal preservation noted in the WHCO2 group is unknown, but similar advantages in regards to reduced adhesions and tumor implantation may be seen. Further studies are recommended to further evaluate this potential benefit.

We used the CMPS-SF to assess postoperative pain which has been well established as a repeatable and reliable method of pain assessment in veterinary medicine. Fransson et al. reported on the CMPS-SF in dogs undergoing laparoscopic ovariohysterectomy using either STCO2 or lift laparoscopy. They found no difference at any time point postoperatively with moderate correlation to aesthesiometry (r= -0.492). In the human literature there are conflicting results as to the potential analgesic effects of WHCO2. There are several studies documenting the reduction of pain in surgical patients with the use of WHCO2 compared to that of STCO2, however there are many in which no difference was found. In a randomized clinical trial in 53 women
undergoing laparoscopic gynecological surgery, WHCO₂ resulted in a trend toward a higher postoperative pain score and was associated with less patient satisfaction.⁶⁹

A potential limitation of our study is that the mild hypothermia seen may not have been sufficient to observe significant differences in the study outcomes. However, studies suggest that any degree of hypothermia < 37 °C can be sufficient to observe impaired hemostasis,¹¹,¹⁵ making the investigators believe that this mild hypothermia is appropriate for the study design. Additionally, the mild hypothermia expected in this study is similar to that observed in most hospitals and is more clinically relevant than creating moderate to marked hypothermia. Another potential limitation is the lack of differentiation between the heating and humidification of CO₂ as a variable. WHCO₂ (37 °C, 98% relative humidity) was compared directly to STCO₂ (22 °C, 0% relative humidity) without a warmed CO₂ (37 °C, 0% relative humidity) or a humidified CO₂ (22 °C, 98% relative humidity) treatment group. The omission of these treatment groups was based on previous work that found warmed CO₂ and humidified CO₂ as individual insufflation treatments were of limited benefit in preventing hypothermia and may even result in greater postoperative pain in humans.⁷⁰,⁷¹

The volume of CO₂ used in our experiment was small in comparison to clinical cases and that reported in the human literature.⁹,³⁷ Given the proposed primary method of laparoscopic hypothermia is evaporation,⁸,⁷² the greater the number of CO₂ particles contacting the peritoneal surface, the more heat loss can be expected. Therefore a limitation of our study was this low volume CO₂ insufflation and perhaps the model would have benefited from the addition of a controlled leak.

The serum concentration of IL-6 was not detectable in any of our female patients and as stated earlier this is likely due to a technical error. Multiple commercial canine ELISA kits are available and the perhaps the use of a more validated kit would have produced detectable results.⁷³,⁷⁴ Another option would have been to record IL-6 concentrations from the peritoneal surface using a small volume of sterile saline to perform a peritoneal lavage.⁷⁵

Block randomization was not performed and unfortunately this lead to an uneven sex distribution. Given our small sample size and inherent risk for type II error, it would potentially have been advantageous to limit our findings to one sex (females). While sex
interactions are included in the results for completion, it is the author’s opinion that any sex effect is likely due to type I error.

In conclusion these data suggest there is no cardiorespiratory or thermoregulatory benefit in the use of WHCO$_2$ for pneumoperitoneum in healthy, mature, dogs undergoing laparoscopy. Some advantages may exist for local peritoneal desiccation and associated complications related to tumor cell implantation and port site metastasis.$^{61-64}$ Further study is required prior to implementation in clinical cases.

2.7 - Footnotes

a. Microsoft Excel, Microsoft, WA, USA
b. BD, Franklin Lakes, NJ, USA
c. Sandoz, Boucherville, QC, CA
d. Fresenius Kabi, Toronto, CA
e. Zoetis Inc, Parsippany, NJ, USA
f. S/5 Anesthesia monitor, GE Healthcare, Madison, WI, USA
g. DOT-34 NRC 300/375 M1014, Datex-Ohmeda Division, Helsinki, Finland
h. S/5 Aespire 7900 ventilator, GE Healthcare, Madison, WI, USA
i. Bair-hugger, 3M, St. Paul, MN, USA
j. Acklands Grainger, ON, CA
k. Baxter Corporation, Mississauga, ON, CA
l. Mila International Inc, Florence, KY, USA
m. LiDCO Ltd, London, UK
n. Flow through cell electrode assembly, LiDCO Ltd, London, UK
o. ABL90 Flex Blood Gas Analyzer, Radiometer, Brea, CA, USA
p. 0.15 mmol/mL, LiDCO Ltd, London, UK
q. 6 mm Laparoscopic cannula, Karl Storz Endoscopy, CA, USA
r. Lexicon Medical, St. Paul, MN, USA
s. Endoflator, Karl Storz Endoscopy, CA, USA
t. Laparoscope, Karl Storz Endoscopy, CA, USA
u. TEG 5000 Thrombelastograph Hemostasis Analyzer system, Haemonetics, Braintree, MA, USA
v. Kaolin, Haemonetics, Braintree, MA, USA
w. 5 mm Round Cup Biopsy Forceps, Karl Storz Endoscopy, CA, USA
x. Emitech K550 sputter-coater, Ashford, Kent, UK
y. Hitachi S-570 scanning electron microscope Hitachi High Technologies Inc.
   Tokyo, Japan
z. Pfizer, New York City, NY, USA
aa. Metacam, Boehringer Ingelheim, Burlington, ON, CA
bb. PROC GLM, SAS, version 9, SAS Institute Inc, Cary, NC

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CHAPTER III: Summary and conclusion

3.1 - Overview and rationale for the use of warmed and humidified carbon dioxide insufflation during laparoscopy in healthy mature dogs

Laparoscopy has become the primary surgical approach for many procedures in both the human and veterinary fields.  

There are many proven benefits to a laparoscopic surgery such as improved visualization, reduced postoperative discomfort, and shortened convalescence times. Human medicine has lead the way in pioneering this modality, and as such continues to search for methods to further refine these techniques. Many surgeons have questioned the use of gas to create a working space, while others have queried the use of CO₂ as the gas of choice for establishing a pneumoperitoneum. Alternative options to create a working space with in the abdomen have been evaluated in human and veterinary patients, however CO₂ remains the safest and most cost effective.

Capnoperitoneum is known to cause a variety of less desirable physiological consequences including reduced venous return, reduced pulmonary compliance, local and systemic acidosis, peritoneal irritation, and peritoneal morphological changes. The focus of research performed by Ott and colleagues, has been to evaluate the contribution of CO₂ insufflation on evaporative cooling, hypercapnia, acidosis, and peritoneal inflammation. It is suggested that perioperative hypothermia is exacerbated by the energy required to saturate STCO₂. The warming and humidification of insufflated CO₂ was proposed to minimize this heat loss. As less water is lost from the peritoneal tissues WHCO₂ is thought to have additional benefits on the local tissues beyond thermoregulation. WHCO₂ has been found in some studies to reduce mesothelial desiccation, and peritoneal irritation, which are known contributors to adhesion formation and postoperative pain. Despite this body of research to date In vitro, research animal and randomized human clinical trails have failed to show consistent thermoregulatory, or analgesia benefits in the use of WHCO₂ over STCO₂.

The objective of our study was to evaluate the effect of WHCO₂ pneumoperitoneum on core body temperature, cardiorespiratory variables, thromboelastography, systemic inflammation, peritoneal response, and postoperative pain in healthy, mature, dogs undergoing laparoscopy. Our null hypothesis was that no
difference in any of these variables would be found. While our findings did reveal some statistically significant differences between STCO\textsubscript{2} and WHCO\textsubscript{2}, these differences were minimal and considered clinically irrelevant.

Mean core body temperature was significantly lower in the WHCO\textsubscript{2} group compared to the STCO\textsubscript{2} group across all time points, contradicting previous reports and suggesting a negligible thermoregulatory benefit in WHCO\textsubscript{2}. The reason for this could be three-fold: 1) Insufflated CO\textsubscript{2} may be rapidly equilibrated in the peritoneal environment to physiological core body temperature and humidity with minor energy expenditure resulting in no advance in WHCO\textsubscript{2}, 2) Total peritoneal surface area in dogs may be significantly less than humans therefore the warming of this surface will provide less protection and 3) Total CO\textsubscript{2} volume insufflated in our study was significantly less than a typical human laparoscopic procedure,\textsuperscript{45} and well below the minimum volume of 4800L is required to cause a drop by 1°C in a 70 kg person.\textsuperscript{46}

Abdominal insufflation is associated with reduced venous return,\textsuperscript{47–49} however in our study no negative effect on CO or CI was evident with pneumoperitoneum. CO and CI actually increased and remained elevated for the duration of the procedure. This is due to the concurrent increase in heart rate and SVI, as a result of the cardiostimulatory effects of an increased intra-abdominal pressure and nociception. An artefactual increase in preload or CVP was thought to be owing to transmission of intra-abdominal pressures across the diaphragm. An increase in SVR was observed in male patients treated with WHCO\textsubscript{2} and inversely in female patients treated with STCO\textsubscript{2}. The cause for this sex effect may be due to a slower rate of CO\textsubscript{2} evaporation with less subsequent hypercapnia and vasodilation. MAP and DAP were marginally elevated in patients treated with STCO\textsubscript{2} compared to WHCO\textsubscript{2}, which is likely related to an increase in SVR.\textsuperscript{28} Values for DO\textsubscript{2}, VO\textsubscript{2}, and ERO\textsubscript{2} were all increased following induction of pneumoperitonelum and VO\textsubscript{2} was found to be higher in the STCO\textsubscript{2} treatment group. The reason for this treatment effect in our study is unknown, however could be due to an increase in core body temperature.

TEG variables MA, G and CoI significantly decreased in both treatment groups intra-operatively, which is typical with surgical hypothermia.\textsuperscript{50–53} A sex interaction was noted with a trend towards post-operative hypercoagulability in male dogs. This finding
is the opposite of that reported in the human in which females, especially pregnant females were found to be more coagulable than male patients due to increased concentrations of fibrinogen, factor VIII and decreases in Protein S. The cause for this sex related difference is unknown but is of limited clinical significance as no values were considered outside the reference range. Generally it is the author’s belief that sex interactions found in this study were of limited importance and may be a result of type I error given the limit number of male patients.

Subjective evaluation of peritoneal biopsies did reveal an increase in mesothelial cell loss with exposure to STCO₂ compared to WHCO₂, however the importance of this finding is unknown and no statistical analysis was performed due to the limited number of samples processed. Despite the difference observed in peritoneal morphology, no significant difference in circulating C-reactive protein or IL-6 was reported, suggesting a similar inflammatory response in both peritoneal environments regardless of local cell desquamation.

A significant increase in the number of postoperative pain scores above 0 was recorded in the WHCO₂ compared to the STCO₂ treatment group, suggesting a possible link to the peritoneal morphology observed. This association and its clinical relevance are questionable, as no pain score in either group warranted rescue analgesia.

In conclusion, based on the results of our study and a review of the human and veterinary literature, while the rationale for the use of WHCO₂ insufflation appears sound, no clinically significant benefit can be found in preservation of core body temperature, cardiorespiratory parameters, coagulation, systemic inflammation, peritoneal morphology or patient comfort. Therefore the use of WHCO₂ pneumoperitoneum in healthy, mature, dogs is not warranted.

3.2 - Future investigations

Our study suggested some benefit in the use of WHCO₂ for peritoneal preservation. The influence of STCO₂ associated peritoneal desquamation on patient discomfort, adhesion formation and tumor cell implantation is an area of active research.⁴⁰,⁵⁵–⁵⁸
An important factor to consider is the volume of CO₂ insufflated, which relates to the amount of leakage from port sites. The greater the volume of CO₂ required the greater the likelihood of a negative peritoneal effect. Species differences in peritoneal surface area may play an important role in the significance of this interaction.

Local peritoneal acidosis is associated with peritoneal irritation and at risk for subsequent tumor cell implantation. The authors of this study chose not to measure peritoneal fluid pH due to the feasibility of maintaining a sterile pH probe within the peritoneal cavity while sustaining a pneumoperitoneum. Tumor implantation and port site metastasis is a significant complication associated with laparoscopy. STCO₂ may contribute to metastasis through a “chimney effect” and altered peritoneal morphology potentially exacerbates this risk by providing an exposed tissue bed for tumor cells to implant.

Investigation into the conservation of mesothelial cells in canine capnoperitoneum and its impact on postoperative pain, adhesion formation and the risk of port site metastasis would be an area of further research.

3.3 - References


4.1 - Figures

Figure 1.1 - Normal Thromboelastography trace. The reaction time (R) is the time interval between the start of coagulation and the point where the clot reaches a 2-mm amplitude. The clotting time (K) is the time for clot formation, which is defined as a clot amplitude of 20mm. The alpha angle is the rapidity of fibrin cross linking and the maximum amplitude (MA) is a measure of clot strength. Fibrinolysis (LY30) is reflected as a percentage decrease in the amplitude at 30 minutes.\textsuperscript{81}
Figure 2.1 - Mean HR in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A sex interaction was noted over time ($P = 0.027$) with male patients having an elevation in HR earlier in the procedure than the female patients.
Figure 2.2 - Median CO in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ and with WHCO$_2$ insufflation. A sex interaction was found on treatment group for CO, with male patients having a significantly higher median CO with STCO$_2$ insufflation compared to WHCO$_2$ insufflation ($P = 0.023$). This was not observed for CI ($P = 0.054$) and the $P$ value for CO once adjusted was 0.161.
Figure 2.3 - Mean SVR in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A significant difference in mean SVR was found over time ($P = 0.009$), however no trends could be identified over time and significant confidence interval overlap is noted.
Figure 2.4 - Mean SVR in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ and with WHCO$_2$ insufflation. A statistically significant sex interaction on treatment group was observed ($P = 0.047$) with a lower mean SVR recorded in male patients treated with STCO$_2$ compared to females.
Figure 2.5 - Mean MAP in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. The mean MAP was significantly higher across all time points with STCO₂ compared to WHCO₂ insufflation \((P = 0.006)\). A sex interaction was noted across time for the MAP \((P = 0.029)\) and DAP \((P = 0.039)\) with a more persistent arterial blood pressure elevation above baseline noted in female patients when pneumoperitoneum duration was greater than 15 minutes.
Figure 2.6 - Mean PaCO₂ in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. A sex interaction was observed over time with a significant decline in PaCO₂ within the first 5 minutes of the procedure in female patients ($P < 0.0001$).
Figure 2.7 - Mean pH in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. No clinically significant trends were found in mean arterial pH with an initial increase following induction of pneumoperitoneum \((P < 0.0001)\), followed by a gradual decline over the remainder of the procedure. A sex \((P < 0.0001)\) and treatment \((P = 0.012)\) interaction was found over time, however no individual treatment effect was seen \((P = 0.867)\).
Figure 2.8 - Mean arterial lactate in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. Mean arterial lactate increased over the duration of the pneumoperitoneum ($P = 0.008$) with a treatment effect ($P = 0.040$) evident. A higher mean arterial lactate in STCO₂ compared to WHCO₂ across all time points. A treatment interaction also noted over time ($P = 0.029$).
Figure 2.9 - TEG trace in a healthy, mature, dog undergoing laparoscopy with STCO₂ insufflation 24 hours postoperatively. Sex effects were seen with r time ($P = 0.023$) and CoI ($P = 0.013$) with male patients having a tendency towards hypercoagulability (A - Male, B - Female)
Figure 2.10 - Mean Coagulation index (CoI) in healthy, mature, dogs undergoing laparoscopy with STCO₂ and with WHCO₂ insufflation. An event effect was seen \( (P = 0.001) \) with CoI increasing in the postoperative period. A three-way interaction was found between treatment, time and sex \( (P = 0.024) \)
Figure 2.11 - C-reactive protein (CRP) concentrations in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ and with WHCO$_2$ insufflation. CRP was found to have a significant elevation in the postoperative period compared to intra-operative measurements at 0 and 60 minutes ($P < 0.0001$).
Figure 2.12 - Scanning Electron Microscopy (SEM) image at 10um of peritoneal biopsies taken at 90 minutes in a healthy, mature, dog undergoing laparoscopy with WHCO₂ insufflation. The WHCO₂ appeared to preserve the microvilli and the mesothelial cell layer.
Figure 2.13 - Scanning Electron Microscopy (SEM, 10um) image of peritoneal biopsies taken at 90 minutes in a healthy, mature, dog undergoing laparoscopy with STCO$_2$ insufflation. STCO$_2$ appeared to result in loss of microvilli of mesothelial cells, and mesothelial cell loss.
### 4.2 - Tables

**Table 1.1 - Complications associated with hypothermia**

<table>
<thead>
<tr>
<th>Complications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Armstrong, Roberts. 2005</td>
</tr>
<tr>
<td></td>
<td>Clark-Price. 2015</td>
</tr>
<tr>
<td>Coagulopathy/ Increased transfusion</td>
<td>Schmeid, Kurz, Sessler, et al. 1996</td>
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<tr>
<td></td>
<td>Kettner, Sitzwohl, Zimpfer, et al. 2003</td>
</tr>
<tr>
<td></td>
<td>Rundgren, Engstrom. 2008</td>
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<tr>
<td></td>
<td>Clark-Price. 2015</td>
</tr>
<tr>
<td>Impaired humoral and cellular immunity</td>
<td>Kurz, Sessler, Lenhardt. 1996</td>
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<tr>
<td></td>
<td>Beilin, Shavit, Razumovsky, et al. 1998</td>
</tr>
<tr>
<td></td>
<td>Armstrong, Roberts. 2005</td>
</tr>
<tr>
<td></td>
<td>Beal, Brown, Shofner. 2009</td>
</tr>
<tr>
<td></td>
<td>Clark-Price. 2015</td>
</tr>
<tr>
<td>Prolonged recovery</td>
<td>Kurz, Sessler, Lenhardt. 1996</td>
</tr>
<tr>
<td></td>
<td>Pottie, Dart, Perkins, et al. 2007</td>
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<tr>
<td></td>
<td>Clark-Price. 2015</td>
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</tbody>
</table>
Table 1.2 - Randomized human clinical trials reporting the core body temperature and pain in WHCO$_2$ compared to STCO$_2$ insufflation.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Temperature</th>
<th>Pain</th>
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<tbody>
<tr>
<td>Ott, Reich, Love, et al. 1998</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Mouton, Bessell, Otten, et al. 1999</td>
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<tr>
<td>Nguyen, Furdui, Fleming, et al. 2002</td>
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<tr>
<td>Farley, Greenlee, Larson, et al. 2004</td>
<td>↑</td>
<td>↓</td>
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<tr>
<td>Hamza, Schneider, White, et al. 2005</td>
<td>↑</td>
<td>=</td>
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<tr>
<td>Savel, Balasubramanya, Lasheen, et al. 2005</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Champion, Williams. 2006</td>
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<tr>
<td>Davis, Mikami, Newlin, et al. 2006</td>
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<tr>
<td>Manwaring, Readman, Maher. 2008</td>
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</table>
Table 2.1 - Anesthetic variables in healthy, mature, dogs undergoing laparoscopy with STCO₂ (22°C, 0% relative humidity), or with WHCO₂ insufflation (37°C, 98% relative humidity). The values for all patients, over all treatment groups is reported as no significant difference in the dose of propofol required for induction, $E_T^{ISO}$, time from induction to baseline reading at 0 minutes, surgery time or total anesthesia time. (Mean, 95% CI)

<table>
<thead>
<tr>
<th>Anesthetic variables</th>
<th>STCO₂</th>
<th>WHCO₂</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propofol (ml)</td>
<td>2.2 (1.5-2.9)</td>
<td>2.55 (1.9-3.3)</td>
<td>0.117</td>
</tr>
<tr>
<td>(Mean, 95% CI)</td>
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<td></td>
<td></td>
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<tr>
<td>$E_T^{ISO}$ (%)</td>
<td>1.75 (1.66-1.83)</td>
<td>1.71 (1.64-1.8)</td>
<td>0.189</td>
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<tr>
<td>(Median, range)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Time from induction to baseline (min)</td>
<td>48 (37-58)</td>
<td>52 (42-63)</td>
<td>0.184</td>
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<tr>
<td>(Mean, 95% CI)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Surgery time (min)</td>
<td>107(102-112)</td>
<td>111 (107-116)</td>
<td>0.128</td>
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<td>(Mean, 95% CI)</td>
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<tr>
<td>Anesthesia time (min)</td>
<td>173 (160-187)</td>
<td>181 (167-194)</td>
<td>0.204</td>
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<tr>
<td>(Mean, 95% CI)</td>
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Table 2.2 - Cardiovascular parameters in healthy, mature, dogs undergoing laparoscopy with STCO₂ (22°C, 0% relative humidity), or with WHCO₂ insufflation (37°C, 98% relative humidity). The values for all patients, over all treatment groups is reported as no significant difference was observed in HR, CI, SVI DO₂ or O₂ER between treatment groups.

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Heart Rate (beat/min)</th>
<th>Cardiac Index (mL/min/kg)</th>
<th>Stroke Volume Index (mL/beat/kg)</th>
<th>Oxygen Delivery (mL/min)</th>
<th>Oxygen Extraction Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean, 95% CI)</td>
<td>(Median, range)</td>
<td>(Mean, 95% CI)</td>
<td>(Median, range)</td>
<td>(Mean, 95% CI)</td>
</tr>
<tr>
<td>0</td>
<td>104 (84-125)</td>
<td>112 (92-137)</td>
<td>1.1 (1.0-1.3)</td>
<td>22.3 (17.5-28.5)</td>
<td>6.2 (3.3-9.1)</td>
</tr>
<tr>
<td>5</td>
<td>114 (97-131)</td>
<td>152 (125-185)</td>
<td>1.4 (1.2-1.6)</td>
<td>30.6 (24.0-39.1)</td>
<td>10.7 (7.8-13.6)</td>
</tr>
<tr>
<td>30</td>
<td>122 (106-138)</td>
<td>165 (135-201)</td>
<td>1.4 (1.3-1.6)</td>
<td>31.4 (24.6-40.1)</td>
<td>10.1 (7.3-13.0)</td>
</tr>
<tr>
<td>60</td>
<td>138 (122-154)</td>
<td>159 (131-194)</td>
<td>1.2 (1.0-1.4)</td>
<td>29.9 (23.4-38.2)</td>
<td>12.7 (9.8-15.6)</td>
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<tr>
<td>90</td>
<td>145 (128-161)</td>
<td>168 (138-205)</td>
<td>1.2 (1.0-1.4)</td>
<td>31.5 (24.7-40.3)</td>
<td>10.4 (7.7-13.4)</td>
</tr>
</tbody>
</table>

* Significant difference from Baseline (Time 0)
Table 2.3 - Median and range VO$_2$ in healthy, mature, dogs undergoing laparoscopy with STCO$_2$ (22°C, 0% relative humidity) or with WHCO$_2$ insufflation (37°C, 98% relative humidity).

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Oxygen Consumption (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STCO$_2$</td>
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<tr>
<td>0</td>
<td>1.36 (1.09-1.7)</td>
</tr>
<tr>
<td>5</td>
<td>3.06 (2.45-3.81)*</td>
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<tr>
<td>30</td>
<td>3.25 (2.61-4.05)</td>
</tr>
<tr>
<td>60</td>
<td>3.8 (3.05-4.73)*</td>
</tr>
<tr>
<td>90</td>
<td>3.29 (2.64-4.1)*</td>
</tr>
</tbody>
</table>

* Significant difference from Baseline (Time 0)
† Significant difference from STCO$_2$