Effect of Increasing Intra-Abdominal Pressures on Hemodynamic Variables and Biomarkers of Ischemia and Acute Inflammation in the Horse

by

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Abstract

Intra-abdominal hypertension (IAH) is defined as increased pressure within the confines of the abdominal cavity. As pressure rises, ischemia of the splanchnic organs will result from direct compression of the organs and vasculature. Intra-abdominal pressure (IAP) is also transferred across the diaphragm to the thoracic cavity, resulting in cardiac ischemia and reduced cardiac output. An abdominal compartment syndrome (ACS) develops if pressures are sustained above 20 mmHg, resulting in new or progressive organ failure.

Elevations in IAP can alter other cardiovascular parameters such as central venous pressure (CVP) used to assess fluid resuscitation. Increased intra-thoracic pressure will increase CVP through compression on the vena cavae, as well as the pulmonary tissues, increasing right ventricular afterload. However, venous return is variable and dependent on the IAP, resulting in further alterations of CVP measurements.

Identification of IAH in horses is complicated, as indirect methods of measurement such as intra-vesicular or gastric pressures are not accurate. Current recommendations for monitoring IAP are through direct, invasive measurement from the peritoneal cavity that risks complications including infection, hemorrhage, and trauma to splanchnic organs.

The effects of IAP on the hemodynamics of healthy horses were investigated with a pneumoperitoneum model to create an IAP of 20 mmHg. Our results indicate that CVP

tends to increase as IAP increases up to 12 mmHg, whereas CVP fell as pressures increased further. Femoral venous pressures (FVP) were significantly increased with changes in IAP, and showed excellent correlation with pressures measured directly from the abdomen. Femoral blood flow and femoral vein diameter were not altered.

Serum biomarkers were also measured as an objective method to monitor IAP in the horse. Blood samples were obtained before, during and after abdominal insufflation. Concentrations of interleukin-10, intestinal fatty acid binding protein, and procalcitonin were determined using equine specific ELISA. The tests indicated that biomarkers were measurable throughout the experiment, but were not altered by IAP. The results of this study indicate that FVP may be used as an indirect measure of IAP in horses. However, moderate increases in IAP was not able to produce significant changes in biomarkers of ischemia and inflammation, as measured by commercial ELISA.

This thesis interpolates material from three papers by the author [reference 11, 12, 16]. Chapter 2 uses material from reference [11] and Chapter 3 uses material from reference [12], both coauthored with Reid Hanson. Meanwhile, Chapter 4 is based on reference [16], coauthored with Elizabeth Barrett, Valeria Albanese, and Reid Hanson.

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List of Abbreviations

ACS   Abdominal compartment syndrome

APP   Abdominal perfusion pressure

CI   Confidence interval

CO_2 Carbon dioxide

CVP   Central venous pressure

ELISA   Enzyme linked immunosorbent assay

FVP   Femoral venous pressure

GCSF   Granulocyte colony stimulating factor

IAH   Intra-abdominal hypertension

IAP   Intra-abdominal pressure

IAV   Intra-abdominal volume

ICU   Intensive care unit

IFABP   Intestinal fatty acid binding protein

IL-6   Interleukin-6

IL-8   Interleukin-8

IL-10   Interleukin-10

IVC   Inferior vena cava

IVCP   Inferior vena cava pressure

LPS   Lipopolysaccharide

LOA   Limits of agreement

MAP   Mean arterial pressure

MODS   Multiple organ dysfunction syndrome

PCT   Procalcitonin

PCWP   Pulmonary capillary wedge pressure

TNF-α Tumor necrosis factor alpha

SIRS   Systemic inflammatory response syndrome

WSACS   World Society for Abdominal Compartment Syndrome

Chapter 1

1.1   Introduction

Elevated levels of pressure within the abdomen, or intra-abdominal hypertension (IAH) has been shown to be an independent predictor of morbidity and mortality in human intensive care units.[1,2] Once organ failure is noted, without treatment, mortality from IAH can reach 100%, and the survival rate, even with directed therapy, is only 40%.[3-6] Causes of IAH can include diseases that arise within the abdominal space, including impactions, strangulating lesions, neoplasia and peritonitis. Additional causes include capillary leak syndrome caused by sepsis, systemic inflammation, as well as aggressive fluid administration.[7,8]

Intra-abdominal pressure (IAP) has been identified as elevated in horses with colitis, uroperitoneum, colonic displacement, volvulus of the large colon, hydrops, lymphatic obstruction, and peritonitis.[9,10] While measurement of IAP in humans is readily obtained non-invasively with a bladder catheter, in horses this method and other indirect methods of measurement of IAP have fallen short in terms of accuracy and reproducibility.[11-14] The current recommendation for IAP monitoring in equine patients is with a piezo resistive microsensor catheter or metal cannula placed directly in the

peritoneal space to measure pressure.[15,16] The risks of the procedure have limited the application of IAP measurement for monitoring clinical patients; therefore, a less invasive, indirect method for pressure measurement in horses is sought. This review will focus on the definitions of IAP and its clinical syndromes, the incidence and risk factors for IAH, as well as the methods of measurement of IAP evaluated in horses and other species.

1.2   Intra-Abdominal Pressure

The pressure within the peritoneal space is defined by the contents of the abdomen and the compliance of the abdominal wall.[8] The abdominal cavity is a closed compartment bounded by the diaphragm cranially, the abdominal wall composed of the costal arch and abdominal musculatures ventrally and laterally, the spinal column and musculature dorsally, and the peritoneal reflection at the pelvis caudally. It is filled with solid organs, hollow viscus variably filled with fluid, gas or ingesta, and a small amount of peritoneal fluid. The steady-state pressure within this space, or IAP, can vary based on the individual's anatomical characteristics, including body size, muscle tone or development, as well as disease processes.[2,8] It can also shift with normal respirations as seen during inspiration (diaphragmatic contraction) and expiration (diaphragmatic relaxation).[17]

1.2.1   Normal Intra-Abdominal Pressure

Normal IAP is subatmospheric, or 0 mmHg.[18,19] Resting, static or baseline pressures within the abdomen in hospitalized humans range from a mean of between 2 and 5 mmHg, and can increase up to approximately 10 mmHg in critically ill adult patients.[1,18,20,21] Post-operatively, pressures may range from 3 to 15 mmHg after abdominal surgery, and later studies have linked recent abdominal surgery with increases in IAP.[18,22] Body mass index and sagittal abdominal dimeter are also positively correlated with IAP measurements.[18,23,24] Current guidelines establish normal IAP levels in critically ill humans as between 5 and 7 mmHg, with higher baseline levels (9 to 14 mmHg) for the morbidly obese patient.[20] In animals, baseline IAP is often found to be subatmospheric using direct peritoneal measurement.[25,26]

Importantly, the pressures within the abdomen are not expected to act like a fluid-filled container, with uniform pressures throughout. The pressures within the abdomen have been noted to differ based on the location of the measurement.[27,28] Intra-gastric pressures, intra-intestinal pressures and sub-diaphragmatic pressures differ significantly, unless the abdomen is filled with a large volume of fluid to distribute the pressure equally. This indicates that a single, focal pressure measurement for IAP may not always be valid for the global pressure within the abdominal cavity.[28]

1.2.2   Assessment of Tissue Perfusion with Intra-Abdominal Pressure

Visceral perfusion can be predicted by subtracting the IAP from the mean arterial pressure, to obtain the abdominal perfusion pressure (APP).[29] This pressure is analogous to cerebral perfusion pressure calculations, which are used to determine perfusion of the

contents within the inflexible confines of the skull.[30] The value of APP has been implemented to improve clinical predictions for the effects of elevations in IAP, as an APP of >50 mmHg was found to optimize survival in human surgical and trauma patients.[29] In a mixed group of medical and surgical intensive care unit (ICU) patients, failure to maintain APP above 60 mmHg by day 3 after admission was noted as a significant factor for determining mortality.[31]

1.3   Evaluation of Intra-Abdominal Pressure

Measurement of IAP has been standardized for humans by the World Society for Abdominal Compartment Syndrome (WSACS), to enable comparisons, and to establish guidelines for clinical therapy and standards for research in this field.[7,8,32] Pressure within the abdominal cavity is measured in humans at end-expiration, in the supine position, and in the absence of contractions of abdominal musculature to reduce external influences.[8] Unfortunately, despite these standards, the critical IAP level that leads to organ dysfunction is variable between patients due to differences in anatomy, physiology and comorbidities, and a single cut off for IAP cannot be globally applied. However, the WSACS has put forth guidelines to delineate the severity of changes in IAP to help address monitoring and therapy.[7,8,32]

1.4   Abdominal Compliance

Increased pressure within the abdominal space is similar to other compartmental syndromes, in that the fixed volume of the anatomic compartment limits the expansion of the component within. The abdominal wall and, to a lesser extent, the diaphragm provide some elasticity, but these boundaries still limit the expansion of the abdominal cavity based on their compliance. Compliance is defined as the measure of the ease of abdominal expansion, and is expressed by the change in abdominal volume per change in IAP in ml/mmHg.[7] Normal compliance of the abdominal wall in humans is between 250 and 450 ml/mmHg.[33]

1.4.1   Alterations in Abdominal Compliance and the Effect of Pressure

Unlike the skull, which is a rigid structure, the abdominal cavity has some capability to reshape itself, based on the compliance of the abdominal wall and diaphragm. This expansion is described in three stages.[34-38] In the initial phase, as abdominal volume increases, IAP increases minimally, and this is described as the abdominal reshaping phase. As the abdomen changes from a spherical to a circular shape, the lateral walls stretch sagittally, whereas the rectus abdominus muscle mainly expands longitudinally. During the second stage, called the stretching phase, the rectus expands laterally as well as longitudinal. Finally, in the third stage, called pressurization, increases in intra-abdominal volume will cause dramatic increases in IAP.[34-38] Compliance decreases as volume within the abdomen increases, with an exponential curve representing the relationship between IAP and volume.[39] (Figure 1-1)

The abdominal pressure-volume curve describing the relationship of these variables is curvilinear, starting out relatively flat and then producing an exponential increase in pressure once the critical volume has been reached. ACS is observed due to a shift of the patient to the steep portion of the abdominal pressure-volume curve secondary to a loss of compliance of the abdominal wall. It is difficult, however, to determine the shape of this curve in clinical patients, and to know when the pressure and volume in the abdomen have reached the critical point where compensation can no longer occur. This compliance can be estimated in patients by calculating the delta IAP, which is the difference between the end-inspiratory IAP and the end-expiratory IAP. The larger the delta IAP, the lower the patient's abdominal wall compliance.[33] By dividing the delta IAP by the mean IAP, the abdominal pressure variation can be calculated and expressed as a percentage to further define the compliance of the abdomen.[33]

1.4.2   Alterations in Abdominal Compliance and the Effect of Volume

In addition to intra-abdominal pressure, intra-abdominal volume (IAV) is the second component for calculating abdominal compliance. Resting, static or baseline IAV is determined at baseline without a pathological volume increase or reduction in abdominal wall compliance. In adult humans, this value is approximately 13 liters, and can be assessed using computed tomography.[40] The IAV can be estimated by measuring at approximately at the level of the umbilicus, as the sagittal diameter just above the virtual line between the xyphoid and the pubic symphysis. The potential volume, or abdominal workspace, determines the ability for the abdomen to increase in volume

without large increases in pressure, and is important for surgical procedures such as laparoscopy. Increased amounts of workspace allow for increases in abdominal volume without increases in IAP, and provides movable room within the abdominal cavity during surgical procedures. The maximum stretched volume is the baseline volume plus maximum workspace, that results in maximum stretch of the abdominal cavity. This volume is dependent on both baseline IAP and abdominal compliance.[39,40]

The stretching capacity of the abdominal wall and diaphragm is influenced by body characteristics, including weight, height, body mass index, age, sex, and fat distribution.[40,41] Comorbidities, including obesity, trauma, and capillary leak syndrome, among others, will also negatively impact compliance.[40,41] Because the pressure-volume curve is not linear, but exponential, identification of where the patient is on this curve will significantly affect treatment and response to therapy.

1.5   Abdominal Compartment Syndrome

Abnormally elevated IAP is described as intra-abdominal hypertension (IAH). In humans, IAH is defined as sustained or repeated elevations of IAP greater than or equal to 12 mmHg.[7,8] Intra-abdominal hypertension is subcategorized in humans into 4 grades based on the extent to which the pressure increases. (Table 1-1) Significant elevations in pressure can result in hypoperfusion, tissue ischemia, and eventually multiorgan failure.[41-44]

Abdominal compartment syndrome (ACS) is the end result of the natural progression of sustained elevations in pressure resulting in changes in organ perfusion and eventually function. A syndrome similar to ACS was first described in surgical patients that developed oliguria, hypoxia, hypercarbia, high peak inspiratory pressures, along with a tense abdomen.[45] The condition of ACS was further defined by the addition of the requirement for the presence of clinical signs including a distended abdomen, elevated IAP and peak airway pressures, inadequate renal function, and inadequate ventilation.[46] To separate ACS from IAH, additional specifications stated that IAP would be consistently higher than 20 mmHg and that the patient's condition would be complicated by one of the following: peak airway pressure greater than 40 cm H_2O, oxygen delivery index <600 ml O_2 per minute per meter^2, or a urine output <0.5 ml/hour.[47] Others have included the requirements for a persistently low blood pH, labile blood pressure, decreased cardiac output, tachycardia, or oliguria.[48,49]

Abdominal compartment syndrome was eventually defined by the WSACS, combining a numerical value with the clinical consequences of prolonged IAH, as sustained IAP above 20 mmHg, or grade III and IV IAH, accompanied by progressive end-organ dysfunction or failure.[7,8] This syndrome is an all or nothing disorder, and, as such, is not graded. As the abdomen is intimately linked to other abdominal compartments, e.g. the thoracic cavity, pressure within the abdominal cavity can directly transmit to other compartments and vice versa.[50,51] Poly-compartment syndrome has also been recently been described, where two or more anatomical compartments have elevated pressures.[7,50]

1.5.1 Classification of Abdominal Compartment Syndrome

There are three types of abdominal compartment syndrome.[8] Primary refers to causes that are intra-abdominal or intra-pelvic. This form of ACS results from a space occupying or expansive lesion that can include a hemoabdomen, hematoma, peritonitis, ascites, abdominal trauma, gastrointestinal obstruction, or neoplastic mass, among other causes. Primary ACS is common in trauma patients, and in post-operative surgical patients. Secondary causes of ACS include extra-abdominal issues such as hemorrhagic shock, large volume fluid resuscitation, or burn injury resulting in whole body anasarca.[3,21] The process for secondary ACS is likely related to an ischemia and reperfusion injury, primarily affecting the gastrointestinal organs.[52] Tertiary ACS results from a recurrence or redevelopment of ACS after resolution of a previous case of primary or secondary ACS. A common cause of recurrent ACS is aggressive closure of an open abdomen in an edematous patient.[21,53]

1.5.2 Incidence of Abdominal Hypertension and Compartment Syndrome

The incidence of IAH in human intensive care unit (ICU) patients has been reported as high as 58.8% in the first week of an ICU stay, and has been identified as an independent predictor of mortality (relative risk 1.85; CI:95%, 1.12-3.06).[1,2] The prevalence of ACS is approximately 0.5% to 8.2% in human patients, with trauma patients having increased rates of diagnosis, between 6% and 14%.[2,32,52,54] A higher incidence has been noted in burn patients, with up to 20% of patients developing ACS in one report.[32] Mortality is especially high in cases of secondary ACS, reported to be 38%

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in trauma surgical patients without abdominal injuries, and 100% in those not related to trauma that were aggressively resuscitated.[3] Survival rates in humans have significantly improved since the recognition of the syndrome, and with the publication of established guidelines for monitoring as well as treatment of elevated IAP.[55] Survival of ACS is currently stated to be 40% with aggressive monitoring and decompression. [4-6]

1.5.3 Risk Factors for Abdominal Hypertension and Compartment Syndrome

Increased IAP is the consequence of an increase in the volume of abdominal contents, a decrease in abdominal wall compliance, or a combination of these two factors. Regardless of the cause of IAH, the inciting event leads to capillary leak syndrome, extravasation of fluid into the interstitial tissues, and tissue edema. This edema results in further increases in pressure in the abdominal cavity, decreasing perfusion and causing restriction of ventilation which hampers oxygen distribution.[21]

Risk factors for increased IAP, which can progress to IAH and ACS, include diseases and disorders that reduce abdominal compliance, increase the volume of the abdominal or gastrointestinal luminal contents, and miscellaneous issues including sepsis, obesity, and body position. Causes of increased IAP can include abdominal surgery, ileus, pulmonary, renal, or liver dysfunction.[1] The severity of abdominal trauma, serum lactate levels, and temporary abdominal closure are independent predictors of survival in abdominal trauma patients with ACS.[56] Additional risk factors include hypothermia, acidosis, anemia, oliguria, high volume resuscitation, and alterations in end-tidal carbon

dioxide tension.[54] The risk factors for IAH and ACS in humans have been well described, and are supported by primary literature.[7] (Table 1-2)

Abdominal wall compliance has been noted to be reduced by burn injuries, especially circumferential burn eschars, and up to 70% of burn patients will develop transient IAH during treatment.[6] Many of these burn patients will become septic, which can increase production of inflammatory mediators including interleukin 6 (IL-6), interleukin 8 (IL-8) and tumor necrosis factor alpha (TNF-α) that act on the endothelial cells in the capillaries to increase leakage of fluid and edema.[57,58] Increased vascular permeability, ileus, and risk factors including excessive fluid resuscitation and disseminated intravascular coagulation all can contribute to IAH in septic patients.[59] In a recent study, 76.5% of septic patients developed IAH, based on mean IAP values. Surgical patients are at increased risk (93% versus 73%) and maximum IAP was greater in non-survivors in septic shock. IAH reduces IAP, increases lactate and creatinine, and requires increased use of vasopressors and mechanical ventilation.[60]

Intra-abdominal volume can be elevated secondary to bowel edema caused by large volume fluid resuscitation often used to reestablish circulating plasma volumes. Crystalloid administration guided by measurement of preload using central venous pressure (CVP) or pulmonary capillary wedge pressure (PCWP) is currently recognized as the standard of care in goal-oriented shock resuscitation in North America.[61-65] There was initially a theory that increased IAP would falsely elevate PCWP, indicating that the goal of resuscitation would be to improve preload to supernormal levels that were more closely aligned with true PCWP.[66] However, crystalloid volume has been shown to be an independent predictor of both primary and secondary ACS,[54] and a positive fluid balance

at 12 hours and 4 days correlated significantly with mortality.[67] Resuscitation to supernormal levels for improvement of the oxygen delivery index, which requires higher volumes of fluids, increased the risk of IAH, ACS, multiorgan failure and mortality in human patients treated for shock, especially blunt force trauma.[68] What was observed was that the development of ACS is an early phenomenon, within the first 3-6 hours after admission, indicating that a 24-hour assessment of fluid balance is often too late.[54] These patients are easily identified, as they had high PCWP, but without a corresponding increase in cardiac index.[69] This indicates that response to therapy is a better tool than arbitrary ranges for IAP. Crystalloid infusions can reduce intestinal perfusion through the development of bowel edema secondary to capillary leak increase hydrostatic pressure and decreased colloid oncotic pressure.

1.6 Physiologic Consequences of Increased Intra-Abdominal Pressure

The secondary effects of elevated pressure within the abdomen can be local, related to the direct effects of compression of the abdominal organs and their vasculature, as well as systemic related to reductions in perfusion as a result of decreased cardiac output.[70]

1.6.1 Cardiovascular Complications of Increased Intra-Abdominal Pressure

1.6.1.1 Effect on Cardiac Preload

Increases in IAP as little as 10 mmHg can significantly reduce inferior vena cava (IVC) blood flow, and therefore cardiac preload, though compression of the abdominal vasculature.[70-76] The reduction in venous return from below the diaphragm is affected in a pressure-dependent manner; as IAP increases, venous return decreases. Cardiac preload can also be reduced by intra-thoracic pressure from cephalad movement of the diaphragm in response to elevated IAP.[31,77] As ventricular filling pressure increases, cardiac compliance is reduced.[71,73,77,78] Cephalad deviation of the diaphragm also narrows the IVC as it passes through the diaphragm, further reducing venous return.[31,79]

The third factor that can affect cardiac preload is the concept of the vascular zone system, which states that IAP paradoxically increases venous return if transmural IVC pressure at the thoracic inlet is higher than the critical closing transmural pressure, known as a zone 3 abdomen. In this state, the abdomen functions as a capacitor, according to Starling's law.[78] This condition is often noted in hypervolemic patients with high IAP. However, if transmural IVC pressure at the thoracic inlet is below the critical closing pressure, called a zone 2 abdomen, venous return is reduced, resulting in pooling of blood in the lower extremities and decreased stroke volume.[31] This is most common in hypovolemic patients, and those in cardiovascular shock.[79,80] Therefore, cardiac preload in IAH is also dependent on the fluid balance of the patient.

1.6.1.2 Effect on Cardiac Contractility

Increased intra-thoracic pressure can also affect cardiac contractility, shifting the Frank-Starling curve down and to the left. Compression of the pulmonary parenchyma

increases both pulmonary arterial pressure and vascular resistance, and reduces left ventricular preload by physically obstructing blood flow to the left side of the heart.[31] As left ventricular output decreases, systemic hypotension and reductions right coronary arterial blood flow can develop, causing ischemia and inhibiting right ventricular contractility. Changes in the pulmonary vascular resistance also result in increased right ventricular afterload, dilating the right ventricle and increasing myocardial oxygen demand.[76,81] The increased risk of myocardial ischemia, as well as physical compression of the left ventricle through the septum, can further decrease cardiac output.[82]

1.6.1.3 Effect on Cardiac Afterload

Systemic vascular resistance can increase by direct compression on the aorta and systemic vasculature, or as autonomic compensation for reduced venous return and decreased stroke volume.[31,82] The compensatory increase in vascular resistance can maintain a stable MAP in the early stages of IAH in the face of decreased venous return. It can also counter reduction in cardiac output in patients with adequate cardiac function and intravascular volume.[82-84]

1.6.2 Respiratory Consequences

The effects on the respiratory system are mainly mechanical. As the thoracic and abdominal cavities are linked by the shared diaphragm, approximately 50% of the pressure within the abdominal cavity is transmitted across and into the thoracic space.[85-87]

Increased intra-thoracic pressure causes an increase in alveolar pressure, increased dead space, and an increased shunt fraction.[71] Transpulmonary pressures, functional residual capacity and static compliance of the chest wall all decrease.[88,89] Ventilation continues, but with an increased level of airway pressure. The results of elevations in IAP is hypoxemia and hypercapnia, beginning at pressures in the abdomen as low as 15 mmHg.[90]

1.6.3   Neurologic Consequences

Intracranial pressure and IAP are linked directly through the cardiovascular system, as well as mechanistically in their physiologic reactions to changes in pressure.[42,91] Increased intra-thoracic pressure can result in a functional obstruction of cerebral venous outflow. In addition, as systemic blood pressures decrease with reduced cardiac output, both have the effect of reducing cerebral perfusion pressure, defined as mean arterial pressure (MAP) minus intra-cranial pressure.[42,92]

1.6.4   Renal Consequences

Elevations of IAP cause both direct and indirect reductions in renal venous and arterial blood flow through compression of the vasculature, as well as the organ parenchyma.[93] Pressures in the abdomen as low as 20 mmHg were noted to reduce renal blood flow and glomerular filtration rate to less than 35% of normal values. This may be attributed to a secondary to a local vasoconstrictive phenomenon as renal vascular

resistance was observed to increase 15 fold.[93] Oliguria develops at IAP levels above 15

mmHg, and anuria can occur at 30 mmHg in normovolemic patients.[44,94]

In IAH, filtration gradient is likely the most appropriate parameter to explain

acute kidney injury, calculated as the glomerular filtration pressure minus the proximal

tubular pressure. This filtration gradient can be estimated by MAP minus 2 times the

IAP.[95] As abdominal pressures play a significant role in renal function and urine

production, oliguria is often one of the first visible signs of IAH.[82] The odds ratio for

renal impairment was noted to be elevated in two separate studies of surgical patients

with increased IAP ranging in severity up to a mean of 12.4 mmHg (range of 3.8 to 41.7

mmHg) in patient post-laparotomy.[44,96] In these clinical reports, IAH was identified as an

independent risk factor for renal impairment.[44,96] In addition, an increasing IAP was

noted to be an early predictive marker of post-operative renal complications.[97]

1.6.5   Effects on Intra-Abdominal Organs

In IAH, blood flow is reduced to almost all intra-abdominal and retroperitoneal

organs, although the adrenal glands are spared.[41,73] Contributing factors can include direct

mechanical compression of the vasculature, myogenic reflexes and mesenteric

vasoconstriction secondary to local hormonal control.[19,98,99] It has been demonstrated that

pressures as low as 10 mmHg can reduce gastric and microvascular perfusion, and

pressures of 15 mmHg can cause marked reductions in splanchnic blood flow, to 40% of

normal levels, with reductions magnified in the gastrointestinal system compared to the

simultaneous effects noted in other organ systems including the heart.[100-103]

Visceral ischemia may also serve as the second hit in the model for multiple organ dysfunction syndrome (MODS), through the development of intestinal edema, ischemia, and bacterial translocation into the bloodstream.[104] An increase of only 20 to 25 mmHg for 60 minutes was capable of reducing mesenteric blood flow by 63%, and resulted in bacterial translocation to the lymphatic system in rats.[41] Maintenance of arterial blood flow at normal levels was unable to offset the reduction in mesenteric blood flow.[41,100,105] Hypovolemia and hemorrhage may exacerbate these effects.

1.6.6 Hepatic Consequences

Hepatic arterial blood flow can be reduced at an IAP of only 10 mmHg; however, changes in portal vein flow were not seen until pressure in the abdomen reached 20 mmHg.[105,106] Direct compression of the IVC may also result in a Budd-Chiari-like syndrome and acute hepatic ischemia. These changes could occur even with the support of blood pressure by fluid administration. While liver dysfunction and IAH have not been directly linked, the severity of IAH can be associated with changes in serum bilirubin, and liver function tests have been affected by increased abdominal pressure.[107-109] In neonates, hepatic oxygenation is reliant on portal vein blood flow, and increases in IAP will quickly result in hepatic ischemia due to compression of the vessels as well as increased oxygen extraction by the gastrointestinal tract.[103]

1.6.7 Effects on Wound Healing

Studies in a canine gastrotomy model noted the negative consequences of an increased IAP of only 12 to 14 mmHg for less than 120 minutes duration after surgical stapled repair of the stomach.[110] In these dogs, fibroblast migration tended to be reduced, and edema and incisional congestion was significantly increased in the incision lines in dogs subjected to this increased pressure. These changes resulted in significant differences in incisional re-epithelialization and healing of the enterotomies. These alterations in healing were increased as the time the abdomen was subjected to increased IAP was extended.

1.7   Measurement of Intra-Abdominal Pressure

Prior to 1911, IAP was considered to vary based on the methods used to measure it, and there was no consensus about the reasons for these discrepancies. A general review noted 10 authors that found the pressure to be atmospheric or subatmospheric, whereas 18 authors noted positive pressure in the abdomen, or that pressure varied with changes in body positon.[111] Measurements of IAP were initially suspected to be positive or atmospheric, and the magnitude of pressure at various levels within the abdominal cavity were noted to be related to the height of a hydrostatic column of the abdominal contents above the point of measurements.[112] It was believed that pressure within the abdominal cavity was relatively non-compressible, hydraulic and hydrostatic in nature, behaving in accordance to Pascal's law. Therefore, pressures obtained at one point in the abdominal cavity were trusted as representative of pressure throughout the abdomen.[8,113] Contradictory to that belief, later investigations using balloons placed at various positions

throughout abdominal cavity and stomach noted variations in pressure changes based on the site of placement, rather than the position of the animal.[28] However, it was noted that filling the abdomen with saline equilibrated the pressure throughout the abdominal cavity.

Measurement of IAP was eventually defined as mainly affected by three factors: gravity, uniform compression, and shear deformation.[114] Uniform compression, including that imposed by abdominal contractions, diaphragmatic contraction, mechanical ventilation, rib cage excursion, and abdominal binding or bandaging, all result in spatially homogenous changes in pressure that can be superimposed on the gravitational pressure gradient vectors.[20] (Figure 1-2) However, shear deformation is dependent on the consistency or compressibility of the tissues and the degree of deformation, and has spatially diverse pressure gradients. These factors (gravity, compression and shear deformation) are what defines whether the abdomen behaves in a hydraulic manner, similar to a liquid filled container.[20]

Given the incidence and risk of IAH in clinical patients, the WSACS recommends monitoring IAP if 2 or more risk factors for IAH are present for determination when and if interventions should be made.[7] If IAH is observed, serial measurements are recommended to be obtained throughout the critical stages of the illness.[32] Recently, a revised IAH and ACS management algorithm in human patients has been noted to increase patient survival to discharge from 50% to 72%.[115] The main alteration in the management strategy was to implement serial IAP measurements to detect IAH, with clear management policies to reduce IAP, restore tissue perfusion, and provide early

surgical decompression in those that developed ACS using the standards set by the WSACS.

The gold standard for IAP measurement in humans at this time is an indirect method through a bladder catheter, in the supine position.[8] Other routes, including gastric, rectal, uterine and intra-peritoneal, have also been used.[113,116,117] For validation of a novel technique, the WSACS recommends a measurement bias of <1 mmHg and precision of 2 mmHg, or limits of agreement (LOA) between -4 and 4 mmHg for IAP techniques to be interchangeable using a Bland-Altman analysis.[118] While similarities between techniques may be observed based on this comparison, the fault of this guideline is that it does not allow the investigator to determine which is more accurate. Despite the ease of use, indirect methods will provide both increased bias and less precision than a direct method can. However, they are often selected, as they are less invasive than a direct technique.

1.7.1   External Evaluation of Abdominal Perimeter

Although changes in abdominal volume have been shown to alter the shape and compliance of the abdomen,[34-38] clinical examination or measurement of the abdominal perimeter has fallen short in its ability to accurately estimate IAP in a number of studies.[119-121] For example, IAP was obtained in patients that developed hemorrhagic abdominal effusions secondary to dialysis with end-stage renal disease. In this study, poor correlation was noted between changes in abdominal pressure caused by hemoperitoneum with abdominal circumference.[122] Another study in human trauma

patients showed that while IAH was present in 32% of patients, clinical abdominal examination had a sensitivity of only 40%, specificity of 94%, and accuracy of 77% for diagnosis of this condition.[119] These findings suggest that specific measurement of IAP is necessary for the diagnosis of this condition, and that abdominal circumference cannot substitute for this measurement.

1.7.2 Intra-Vesicular Measurement

Intra-vesicular measurement of IAP remains the reference standard for clinical IAP measurement in humans recommended by the WSACS due to both cost and accessibility.[7,8,32,113] This method is an indirect measure of pressure, which assumes that the pressure with in the abdomen is hydraulic, and is transferred across the wall of the organ to the urine within. The pressure within the bladder is obtained using a bladder catheter, with an instillation volume of saline used to produce a solid fluid column to the system of pressure measurement, typically a water manometer or electronic pressure transducer. The pressures can be obtained via a 16-gauge needle inserted in the aspiration port and are measured in mmHg, at end-expiration, in the supine position, with the transducer zeroed at the midaxillary line. However, this method has a number of drawbacks, as it provides only intermittent monitoring of IAP, interferes with urine output, increases the risk of sepsis and urinary infections, and can increase the risk of needle sticks to care providers.[22,123,124]

Alternatively, a closed system can be used that places a three-way stopcock between the catheter and drainage bag to allow for instillation of fluid as well as

monitoring without disconnection of the urinary catheter distal to the culture aspiration port.[125] It also does not require repeated needle insertions once a catheter is placed in the culture aspiration port, reducing the risk of injury.[22,123] Measurements are obtained by clamping the drainage tubing distal to the catheter. Measurements can be intermittent or continuous through an irrigation port of a three-lumen bladder catheter, and was validated in experimental animal models, in human surgical and trauma patients, as well as in an ICU setting.[117,126,127] This closed method for intra-vesicular pressure monitoring is still a fluid-filled system, with all the problems associated with a fluid system including air bubbles and damping of the pressure. The catheter can kink, leak, and the replacement of the catheter in the aspiration port can increase the risk of infection or needle sticks. However, the method is safer, faster, less invasive than the open technique, and ideal for screening in the short term.[113] Further modification of the technique by connecting the pressure transducer to a third stopcock reduces the risk of leakage and increases sterility.[113]

A simplified approach for intra-vesicular pressure monitoring has been evaluated in both humans undergoing laparoscopic cholecystectomy and a porcine experimental model of IAH using a U-tube technique.[128,129] In this method, the patient's own urine within the urinary catheter serves as the transducing medium. To obtain pressure measurements, the urinary catheter is held above the patient at a 90-degree angle from the body, and the column of fluid measured referenced to the standard reference point in centimeters of fluid in the column. A fluctuation should be noted in the meniscus with respirations to ensure pressures are transferred correctly to the fluid column.

An advantage of the U-tube technique is that it is a closed system, which may reduce the risk of infection. It also is simple, in that it requires no additional equipment or monitors to obtain intra-vesicular pressures. However, it still has the inconveniences and inaccuracies noted in any fluid-filled system. In addition, the volume re-instilled into the bladder when the catheter is elevated is not defined, and may affect pressure measurements if the volume is large. However, pressure obtained in both the porcine and human validation studies were more accurate and precise than those obtained by a standard intra-vesicular technique.[128,129]

A commercial medical device based on the U-tube technique (Foley Manometer, Holtech Medical, Copenhagen, Denmark) has been developed and validated in vitro in an effort to standardize the instillation volume in the system.[130] This device uses a 50 ml container with a biofilter placed between the Foley urinary catheter and the drainage bag. When the container is elevated, a set 50 ml of urine flows back into the bladder, reducing the risk of false elevations by the detrusor contractions. The correlation, precision and accuracy was good when compared to the standard intra-vesicular measurement technique. Disadvantages include the possibility of obstruction of the biofilter, the present of air bubbles in the manometry tube, and the additional cost of the device.

Complications that may lead to invalid intra-vesicular pressure measurements of IAP can include malpositioning of the pressure transducer, artifacts in fluid lines including air bubbles, the compliance of the pressure tubing, and body position of the patient.[113] While the intra-vesicular method of IAP pressure measurement has been recognized as the gold standard for indirect measurement of IAP, its validity has been called into question due to increased inter-individual and inter-institutional variation, and

lack of reproducibility.[89,113,130] Coefficients of variation for intra-vesicular pressure measurements can range up to 66% in some institution, making it difficult to monitor trends in an individual patient, and to compare data between patients and institutions.[2] While methods to measure intra-vesicular pressure have been validated, the comparisons vary widely in regards to the reference point, the fluid instillation volume, the species studied, as well as whether the pressures were monitored in an experimental setting using pressurization with fluid or gas, or in subjects with naturally occurring IAH.[89,123,124,131-134] In fact, the bias and precision guidelines currently set by the WSACS for validation of novel techniques for IAP measurement would exclude the use of intra-vesicular pressure as an indirect measure of IAP if it was validated today, as it could not pass the test for accuracy and repeatability.[118]

The fluid instilled in the bladder as a priming volume has also been demonstrated to affect measurement due to activation of the bladder's detrusor muscle, falsely elevating the pressures obtained.[113,131,135,136] In addition, intra-vesicular catheters may systematically overestimate the pressure in the abdomen, due to intrinsic contractions of the detrusor muscle.[135,136] While a rapid flush test can be performed to determine the dynamic response of the system and tendency of the pressure tubing to over- or under-damp the signal, this test is difficult to interpret.[123] The rate of infusion, and temperature of the fluid used for priming the system also could result in contraction of bladder musculature. Methods to reduce variation can include the use of smaller priming volumes, as volumes higher than 10 ml are known to cause changes in the intra-vesicular pressures obtained.[17,137,138] It is currently recommended that the lowest volume possible be used that will establish a fluid column to the measurement device to reduce the risk of

falsely elevating the pressure, as pressure can be measured in an near-empty bladder.[101] However, extremely low volumes increase the risk of loss of a fluid column and prevent measurement altogether.[139] The current recommendation by the WSACS is to use a maximal instillation volume of 25 ml in adult humans.[7,32]

Body positioning has been observed to influence IAP measurements obtained by a bladder catheter.[20,140] Based on these findings, measurements are recommended in humans in the supine position. In addition, inspirations will also have an effect. In humans, it is recommended to obtain all vesicular pressures at end-expiration, as this is the point in the respiratory cycle when the abdominal musculature contractions are absent. It is also least likely to be affected by the mechanics of positive pressure ventilation, which is commonly performed on critically ill patients.

Additional complications of indirect measurement of IAP using an intra-vesicular catheter is that trauma or diseases that occur in the caudal abdomen or pelvic canal can affect measurements. The effect of intrapelvic masses on pressure has been termed intrapelvic compartment syndrome, and may be an explanation for erroneously increased pressure measured from the bladder.[116] Examples of situations that may alter intra-vesicular pressure measurements include patients undergoing damage control laparotomy, the use of abdominal packing to control hemorrhage, the presence of pelvic hematomas, or urinary bladder rupture.[141] As pelvis fractures, as well as rupture of the stomach or urinary bladder, are commonly seen in the emergency department secondary to trauma, these injuries could hinder the usefulness and interpretation of bladder pressure measurement for monitoring IAP.

Anatomical variations also complicate the accuracy of intra-vesicular pressure measurements, specifically in regard to the abdominal location of the bladder. In humans, the bladder is described to be retroperitoneal, thereby occupying a compartment separate and external to remainder of the abdominal cavity, whereas the bladder in pigs, dogs, and horses is known to be positioned within the peritoneal cavity.[136] These species differences may make measurements of bladder pressures variable and the method used to obtain and interpret the pressures species specific.

1.7.2.1 Measurement of Intra-Vesicular Pressure in Small Animals

Although dogs have been used extensively in the experimental evaluation of intra-vesicular pressure as an indirect measure of IAP, there are few reports of pressure measurement in small animal veterinary patients. In dogs, the reference interval has been provided as 0 to 7.5 cm H_2O. [142]

In healthy cats, a reference interval has been described using intra-vesicular pressures under both anesthesia and in awake cats.[143] The cats were anesthetized with propofol, and a 5 French catheter placed in the urinary bladder with radiographic guidance. The pressures were measured using an open technique, employing two stopcocks within the system to allow for emptying of the bladder, instillation of the residual volume, and measurement of pressure with a water manometer. An instillation volume of 0.5 ml/kg or 1 ml/kg was instilled, and the manometer referenced to the pubic symphysis. Measurements were obtained in triplicate, and performed in sternal and lateral recumbency. The measurements were repeated in the awake cats.

The results of these measurements found that intra-vesicular pressures in clinically healthy cats produced a mean pressure of 7.0 cm H_2O (5.23 to 8.83 cm H_2O). The IAP varied widely, and the pressure were significantly higher in awake cats. The stress of manipulation likely produced both changes in body position as well as muscle contractions in the body wall and bladder causing the pressure to spike. Body position was observed to affect measurements, as IAP was higher in right lateral recumbency than in sternal recumbency. This is similar to observations in humans, and can be related to the weight of the organs, the organ content and density, and the position of the external reference point of measurement. In humans, supine positioning is recommended, but this position is unnatural in most veterinary species, especially in awake quadrupedal animals. Bladder instillation volume also increased IAP as volumes increased, similar to humans. The volume chosen for this study was arbitrary, therefore the ideal volume could not be determined for pressure measurement. Finally, body condition score also influenced intra-vesicular pressures, as a score of 7 was significantly increased compared to body condition score of 5 and 6. A wider range of body conditions would be necessary in the cat to determine if obesity played a role in effects on IAP.

Measurements of intra-vesicular pressure in these cats were not compared with direct measurement of IAP or other measures of IAP; therefore, it is not possible to gauge the accuracy or precision of these measurements against a control. The system used, the number of cats, and the health status of the cat, all may provide some variation when this method is pursued in clinical cases. Geriatric, pediatric, and clinically ill patients would further help to define the range of normal pressures in the cat, and provide guidance as to abnormalities seen in clinically ill patients.

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In a separate study, intra-vesicular pressures were obtained in cats undergoing ovariohysterectomy, both pregnant and non-pregnant, as well as tomcats undergoing routine orchiectomy.[144] Pressures from the bladder were obtained using a closed system, similar to the method of Radar and Johnson, and the bladder emptied prior to obtaining the measurements.[143] An infusion volume of 0.5 ml/kg was used and the measurements were observed in lateral recumbency with the reference at the pubic symphysis. Pressures significantly increased in both pregnant queens and tomcats immediately after surgery, demonstrating an effect of the procedure, however specific values were not provided. Intra-vesicular pressures were stated to be similar to previous studies.[143] Pregnancy in its early stages did not alter baseline or post-operative IAP compared to non-pregnant queens and tomcats.

In dogs, IAP has been observed in clinically normal females before and after ovariohysterectomy as a control group, as well as in a cohort group with evidence of gross abdominal distension.[142] The closed technique was used, with a water manometer directly attached to the urinary catheter via a three-way stopcock and the bladder was continuously emptied between measurements.[123] Pressures were obtained in lateral recumbency after sedation for the surgical procedure, and in awake dogs after surgery, as well as in the cohort group. In the control group, preoperative pressures averaged 4.5 +/- 0.44 cm H_2O, and ranged from 0 to 15 cm H_2O. Postoperatively, pressures were increased in the dogs undergoing surgery, with a mean of 7.51 +/- 0.45 cm H_2O, and remained elevated for the 24 hours of the study, suspected to be secondary to abdominal splinting, edema and abdominal inflammation. In the cohort group, diseases observed included gastric dilatation and volvulus, closed pyometra, hemoperitoneum, ascites and

diaphragmatic herniation. In the animals with gross abdominal distension, all had peak pressures greater than 16 cm H_2O, and pressures over 30 cm H_2O were associated with the development of anuria in two dogs. Surgical intervention resolved the IAH. Complications of the technique included one case of a urinary tract infection.

Similar results were observed in a prospective study involving dogs admitted to an intensive care unit allocated into two groups based on the risk factors for IAH in humans identified by the WSACS.[8,145] These animals were monitored with a closed-system commercial intra-vesicular pressure monitoring kit (AbViser™ Intra-Abdominal Pressure Monitoring System, ConvaTec, Skillman, NJ).[146] Pressures were obtained in lateral recumbency, referenced to midline, and used an instillation volume of 1 ml/kg of saline. Risk factors for IAH identified in these dogs included abdominal trauma, ascites, abdominal compression, abdominal surgery, acidosis and vasculitis. Intra-vesicular pressures in the dogs at risk for IAH were significantly higher (9.4 +/- 3.4 cm H_2O) than those without risk factors (4.1 +/- 0.9 cm H_2O). Bland-Altman plots were used to identify inter- and intra-observer variability, which was low, and observations correlated well between observers. These reports provided evidence to help establish the intra-vesicular pressures in awake dogs in the intensive care unit; however, the intra-vesicular pressure was never compared to direct measurements to determine precision or accuracy.

Pressures were assessed in an experimental protocol evaluating the effect of open and laparoscopic abdominal procedures as well as the intra-vesicular instillation volume of saline in healthy dogs.[147] Instillation volumes assessed ranged from 0.25 ml/kg to 2 ml/kg. The measurements were performed in lateral recumbency as described by Conzemius et al, and the reference point was defined as the pubic symphysis.[142]

Correlations between laparoscopic pressures and intra-vesicular pressures were best when a volume of 1 ml/kg was instilled. However, pressures obtained at baseline under sedation were not altered by bladder instillation volume, consistent with reports that noted fluid volume instilled has a larger effect on intra-vesicular pressure measured at higher levels of IAP.[136] Mean baseline pressures in these dogs were higher than other reports (mean pressure of 8.7 +/- 4.4 cm H_2O) emphasizing the variability in this technique across populations and investigators, as well as the need for standardization of outside influences to provide reference intervals for clinical patients.

1.7.2.2 Measurement of Intra-Vesicular Pressure in Horses

Standardized comparisons of intra-vesicular pressures to direct peritoneal pressures were first described in the literature in standing, awake horses under sedation.[11] The bladder was catheterized and an open method was used to allow for pressure measurement.[22] The bladder was emptied between measurements, and infused with warm, sterile saline in volumes that ranged from 0 ml (providing only fluid to fill the catheter itself) to 200 ml. Intra-vesicular pressure was obtained at end-inspiration, when abdominal contractions were absent, and the manometer was referenced (zeroed) to the tuber ishii.[148]

In healthy horses, normal intra-vesicular pressures were subatmospheric (-8.63+/- 4.37 cm H_2O). Intra-vesicular pressures demonstrated a large variance, more than 3-10 times that of directly measured IAP. Infusion volumes greater than 50 ml positively influenced intra-vesicular pressures, however infusion volumes of 0 ml sometimes failed

to establish a fluid column to allow for pressure measurement. Head height, which is known to affect mean arterial pressure in standing horse, did not affect intra-vesicular pressure measurements.[149] While pressures were significantly different when compared to direct measures of IAP, this could be attributed to the fact that the bladder pressures were referenced and obtained at a point much higher in the abdomen, which reduced the weight of the viscera on the organ's exterior.[103] The difference may also be related to the effect of regional pressure dynamics, as the abdomen in these healthy horses was not distended with fluid and could not act in a hydraulic manner.[28] The poor correlation with pressures measured directly from the abdomen, and the high variability both would indicate that intra-vesicular pressure measurement is not valid for monitoring IAP in normal, standing horses.

Intra-vesicular pressures were also obtained in horses using a custom air filled bladder validated ex vivo.[13] Pressure within the bladder of standing, unseated horses was obtained by 15 consecutive measurements on two separate occasions. Variability was significant for measurements made within the same individuals, and a correlation could not be observed between pressures obtained from the peritoneal cavity of sedated horses measured on a separate occasion. This research may support the findings of the previous study. However, issues with technique including observations of alterations in pressure due to contraction of the bladder on the balloon, the fact that pressures were not obtained simultaneously, and the use of a novel pressure manometer for this study may hinder the interpretation. Respiratory variations could also not be observed in the manometer during measurement of intra-vesicular pressures, which may indicate damping of the system and inaccurate results.

1.7.3   Direct Measurement of Peritoneal Pressure

Direct measurement of IAP has been described, but has not been widely accepted in human medicine due to the invasive nature of the technique, the cost of additional catheters or abdominal drains, and possible complications including hemorrhage, infection or trauma to the abdominal organs.[19,150] Direct pressure measurement is more commonly used in research and for validation of indirect techniques.[151,152] Pressures may be obtained directly from the abdominal cavity using an intra-peritoneal catheter, or in surgery using a laparoscopic insufflator, which provides continuous monitoring of abdominal pressures.[19,71,72,79,100,123,153]

1.7.3.1 Measures of Direct Peritoneal Pressures in Humans and Animal Models

Comparisons of continuous pressures obtained from a 14 French intra-peritoneal surgical drain and bladder pressures in humans noted a good correlation with laparoscopic pressures (r^2=0.962).[154] Stepwise comparisons were performed at IAP of 5, 8, 12 and 24 mmHg. However, a Bland-Altman analysis was not performed; therefore, systematic bias cannot be determined. The use of drains is uncommon in the majority of human intensive care patients, and the fact that many patients will require monitoring of IAP who do not have drains may preclude the use of this technique.

In an experimental porcine model, direct pressures obtained using a piezo resistive pressor sensor with a unicrystalline semiconductor have been validated against

an intra-vesicular catheter, as well as a laparoscopic insufflator.[155] Pressures were also obtained simultaneously using an air capsule pressure system (RCP-PU/PPUP, Life-Tec, Stafford TX), where an air inflated capsule is inflated to obtain pressure readings as it equilibrates with the pressure surrounding it. The air capsule pressure measurements were not significantly different from the insufflation pressure, whereas the vesicular and piezoelectric measurement were noted to be significantly below the insufflation readings, which contradicted previous reports comparing intra-vesicular pressures to laparoscopic insufflation in humans.[128,131,132] The main difference cited by the researchers for this finding is that the bladder in the pig is intra-peritoneal, which may have collapsed the bladder and occluded the manometer measurement. Similar observations were noted in another porcine study, highlighting the differences between pressure measurements in animals and human patients.[136]

While the piezo resistive probe underestimated the IAP in this study, it was by a small amount, 1.6 +/- 4.8 mmHg, but with wide limits of agreement (-8 to 11.2 mmHg). The authors contributed this to the higher sensitivity of the probe to changes in pressure, as both inspiration and blood pressure were noted to alter IAP obtained by this method. While the air-capsule provide the lowest difference from the insufflation pressure (LOA: 0.5 +/- 2.5 mmHg), these measurements tended to drift, likely due to penetration of the capsule by carbon dioxide, which is highly soluble. Based on these findings, the piezo electric probe was determined to be equivalent to the standard device, while the air capsule catheter was more precise for immediate measurement.[74]

This experimental design was repeated with a second piezo resistive pressure probe, that it could be zeroed while in situ (ACCURATE plus, MIPM, Mammendorf,

Germany).[156] Overall, the piezo resistive probe displayed the smallest mean difference and confidence intervals, indicating improved measurement capability compared to both bladder and a water balloon probe techniques. While capable of measuring IAP, this piezo resistive probe still must be placed within the abdomen, risking fragmentation, and infection. The increased cost of the device should be considered.

A commercial catheter used to monitor pressure in fascial compartments (Stryker intercompartmental pressure monitor, Stryker Corp., Kalamazoo, MI) has been evaluated in humans compared to a laparoscopic model of insufflation.[157] This system involves an indwelling slit catheter, placed with an introducing needle and connected by pressure tubing to the monitor. The probe was zeroed to the pubic symphysis, and comparisons were made up to IAP of 25 mmHg. The mean difference between methods was only 0.04 +/- 3.8 mmHg. The advantage of this system is that pressure changes were obtained in real time, and no delay was noted. Clinical complications were not observed, making this system a viable method for continuous measurement of IAP in clinical patients. Limitations of the technique are that it may require guided insertion to prevent injury to abdominal organs, and that it is by nature an invasive technique. The catheter can also become infected, occluded, and the abdominal organs may occlude the slits in the catheter. In addition, the device's output may drift over time, therefore recalibration is recommended in situ every 6 hours.

1.7.3.2 Measures of Direct Peritoneal Pressures in Horses

Direct measurement of IAP in horses was first reported in two horses with abdominal effusions due to peritonitis.[10] Ultrasound guided needle puncture of the abdomen was performed and the measurements of IAP were obtained from the lateral abdomen using an electronic pressure transducer. Average readings ranged from 17.2 mmHg to 25 mmHg between the two horses. Based on these IAP readings, and the presence of organ dysfunction and cardiovascular abnormalities consistent with human cases of IAH, a diagnosis of ACS was proposed. However, direct comparisons were not appropriate with the classification scheme given for humans, as both normal pressure and the technique for measurement of IAP in the horse had not been established.

Direct measurement of peritoneal pressures was first described in healthy horses combined with validation of intra-vesicular pressure measurements.[11] (See Chapter 1.7.2.2) Pressures were obtained by use of a metal cannula introduced into the abdomen at a point midway between the point of the shoulder (cranial eminence of the greater tubercle) and the tuber ishii, to mimic the mid-axillary line in humans.[8] Pressures were obtained in fasted, standing horses under sedation, using a manometer, closed system pressure tubing and a stopcock to allow for infusion of saline. Pressures obtained were subatmospheric, with a mean of -1.80 cm H_2O (95% CI: -2.8 to -0.8 cmH_2O). As for intra-vesicular measurements, head height did not affect pressure readings, but holding the head in a neutral position provided more consistent measurements of IAP. A separate report comparing gastric pressures to intra-peritoneal pressure using the same methods recorded IAP similar to those found in the previous study (-1.59 +/- 2.09 cm H_2O).[12]

Similar results were obtained in unsedated, healthy horses, where pressures were measured from the left and right flank, using the same reference points as Munsterman,

et. al., in addition to measurements obtained on the ventral midline.[11,158] Pressures at the level of the flank were again subatmospheric, with a slightly lower pressure measured in the right flank (-5 +/- 3 mmHg versus -3 +/- 2.5 mmHg). These pressures were consistent with the previous report, showing little effect of sedation on the IAP values in standing horses.[11] However, the ventral midline pressure was significantly higher (25 +/- 3.1 mmHg) than the flank pressures, consistent with the effect of the weight of the viscera in a standing quadruped.[159] These differences between the flank pressures and ventral midline also may further demonstrate the regional differences in pressure noted in the abdomen in other species.[28] Evaluation of horses under anesthesia, in lateral and dorsal recumbency, further demonstrated the effects of body position and weight of the viscera on direct IAP at different positions in the abdomen.[27] The direct IAP increased when measured in at the insertions sites in flanks, while pressure decreased on ventral midline when horses were in dorsal recumbency. The opposite was observed in lateral recumbency, as ventral midline pressure increased, and the pressure at the cannula insertion sites in the flanks decreased.[27]

1.7.4   Measurement of Gastric Pressures

Gastric pressure measurement as an indirect measure of IAP has been proposed, as it is theorized that the stomach can act in a similar manner to the bladder to transduce the pressure within the abdomen to the fluid within.[113] Indications for gastric pressure measurement of indirect IAP include patients without a Foley urinary catheter, or in patients with complications directly involving the bladder, including trauma, adhesions,

hematomas, pelvic fractures, abdominal packing, or neurogenic dysfunction.[160] Disadvantages of this system are similar to any fluid-filled system. Additional problems may be related to the migrating motor complex activating the gastric musculature, or altered pressures due to nasogastric feedings or oral fluid administration. Measurement of gastric pressure requires the removal of all air from the stomach, and verification of adequate decompression is not possible. However, gastric pressure measurement is cost effective, and the risks associated with bladder measurement are avoided.[113]

1.7.3.1 Measurement of Gastric Pressure in Humans and Animal Models

Gastric pressures were first recorded in humans using a manometer consisting of an air filled balloon to record the changes in abdominal pressure with the respiratory cycle.[161] The balloon method noted slight delays with pressure changes, and an oscillation in the pressures of 2-3 cycles per second was noted on the manometer, suggested to be due to muscular contractions.[162] More recently, gastric tonometry was validated in a porcine model which noted good correlation and a small bias when compared to direct pressure measurement, as well as measurement of IAP from the urinary bladder.[134] Similar findings were noted when compared to pressures obtained during laparoscopic surgery in humans, and the methodology has been validated against direct IAP and intra-vesicular pressure in anesthetized dogs.[163-165] The system was also validated against a tonometer and intra-vesicular measurement of IAP.[113] Although a validated technique, this catheter is more costly, which limits its use in a clinical setting.[128]

A balloon system can be advantageous, as it has little to no infection risk, no interference with urine output, and may allow for measurement of mucosal carbon dioxide concentrations. Both fluid and air are viable for inflation of the balloon, but over inflation may artificially increase IAP.[166] The closed nature of air or fluid insufflation balloons eliminates problems associated with a fluid-filled system in regards to a zero reference, damping and body position. Trends can also be recorded over time.[166] Disadvantages are that the issues with gastric migrating motor complexes remain, and a special syringe is needed to inject air into the balloon. The air can also be absorbed through the balloon, requiring recalibration.[117]

In human patients, gastric pressures have also been obtained using a nasogastric or gastrostomy tube placed in the lumen of the stomach.[45,76,96,160,163] The gastric pressure is obtained by instilling the stomach with 50 to 100 ml of water to produce a fluid column in the tube. The proximal end of the tube is then held perpendicular to the flow, similar to the U-tube intra-vesicular technique, and the pressure is obtained using the midaxillary line as the reference point for the meniscus of the water column. Comparisons of nasogastric catheters with laparoscopic insufflation, rectal pressures using a modified esophageal stethoscope, and a bladder catheter noted that both gastric and rectal pressures were unreliable, with gastric pressure producing a bias of -0.7 +/- 9.8 mmHg.[89] Gastric pressure were position dependent, and the contents of the stomach were thought to influence the measurements. Similar results were shown using simultaneous measurement of IAP in human patients with indwelling urinary and gastric catheters. Using a 50 ml instillation volume, the pressure were obtained at the mid-axillary line. The mean difference was 0.05 cm H_2O (standard deviation: 1.29 cm H_2O; LOA: +/-2.58

cm H_2O).[160] Correlation was excellent and the only complication noted was a falsely elevated pressure caused by coiling of the tube within the stomach.

Continuous gastric pressure measurements would be preferred for clinical use, and have been attempted with semi-continuous readings from a gastric tonometer measured in humans undergoing laparoscopic procedures.[163] The bias between the gastric balloon and bladder pressures was 0.35 mmHg, (LOA: -3.1 to 3.8 mmHg). Similar findings were noted in a study using an air-filled compliance balloon catheter in elective laparoscopic cholecystectomies (bias: 0.122 +/- 0.7).[167] While statistically acceptable, the disadvantage of this system is that it can be affected by migrating motor complexes, which can last for up to 2 minutes at a time.[168] It is also much more expensive to perform depending on the catheter selected. The gas in the balloon can also be reabsorbed in 4-6 hours, therefore recalibration is needed.[117] Complications can include interference by peristalsis, and medications or enteral nutrition administered.

1.7.3.2 Measurement of Gastric Pressure in Horses

Intra-gastric pressures have been assessed in healthy horses, and compared to direct peritoneal measurements of IAP.[12] Pressures were obtained with a U-tube manometer technique using a nasogastric tube, and pressures were obtained using varying gastric instillation volumes, from 0 to 3000 ml. Mean gastric pressure was positive (14.44 +/- 4.69 cm H_2O). Although repeatability was good within horses (variance: 0.34-0.91 cm H_2O) there was an unacceptably large variation between horses, 4 to 10 times that of intra-peritoneal pressures. Volume infused did not significantly affect the

pressures measured from the stomach. The bias was high (15.9 +/- 5.3 cm H$_2$O) and correlation between the 2 techniques was poor (Lin's Concordance Correlation Coefficient: -0.003), indicating the two methods are not interchangeable.

While gastric tonometry has been commonly employed for measurement of gastric pressures studies investigating respiratory mechanics in horses, few have compared intra-gastric pressures to direct measures of IAP.[169-172] An initial study evaluated the effect of gaits and exercise on gastric pressure and IAP.[169] Balloon catheters were used, placed in the intra-thoracic esophagus, the stomach, the rectum, and in the abdominal cavity on ventral midline. Gastric pressures were found to be affected by exhalation during exercise, with increasing speeds resulting in increasing pressure. However, IAP was not significantly altered by exercise, although exhalation pressures were slightly higher than inspiratory pressures. IAP were also found to be positive, consistent with other direct measurement of IAP on ventral midline, but at a much lower pressure than with a fluid system (an average of 2.4 mmHg for inhalation and 3.7 mmHg for exhalation).[27,159] Rectal pressures were higher during expiration at rest, and increased with increasing speeds, with a trot and gallop causing the highest pressures, but during inhalation, not exhalation.[169]

More recently, a gastric tonometer was used to obtain pressures within the abdomen compared to pressures obtained by passive inflation of room air in the right paralumbar fossa.[14] The pressure from the stomach did not correlate with pressure obtained from the peritoneal cavity, however, the authors stated difficulties in calibrating the gastric balloon, as normal oscillations in gastric pressure did not register.[169] Pressures within the abdomen were more negative than other reports in standing horses, but as the

pressures were obtained at a level higher in the abdomen, this could account for the differences.[11,12]

1.7.4 Measurement of Venous Pressure

Femoral venous catheters are often used for administration of drugs, monitoring of central venous pressure and sampling of venous oxygen saturation.[64] Catheter placement in the femoral vein is considered safer than the subclavian and internal jugular vein in regards to complications including carotid and subclavian arterial puncture, cardiac tamponade, hydrothorax, hydromediastinum, infection, brachial plexus injury, Horner's syndrome, phrenic nerve injury, thoracic duct trauma, pneumothorax and pleural puncture.[173-176] Possible complications of femoral venous catheters include thrombophlebitis, deep vein thrombosis, femoral artery puncture, retroperitoneal hematoma, arterial-venous fistula, and catheter related sepsis, but the overall risk is similar to other types of central venous catheters.[173,174,176,177] The risk of sepsis or bacterial colonization rate is not statistically higher for femoral venous catheters than non-femoral catheters.[178,179] Advantages of a femoral venous catheter over indirect techniques are that continuous pressure trends can be obtained, pressure measurement does not interfere with bladder output, and that it can be used in cases where intra-vesicular pressure measurement is not possible.[113]

Venous pressure was historically investigated and linked to cardiac output by Ernest Starling and others.[180-182] It is known that as IAP rises above venous pressures, venous return is compromised, systemic vascular resistance is increased, and tissue

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perfusion subsequently decreases.[4] Experimental investigations in canines have noted inferior vena cava (IVC) pressures measured trans-femorally correlated directly with IAP over a range of pressures studied, suggestive that femoral pressures could substitute as an indirect measure of IAP.[71,93] In a study in rabbits, urinary bladder pressures as another indirect measure of IAP were noted to consistently and accurately represent IAP measured in the IVC over a range of experimentally produced pressures.[133]

However, published data is still conflicted as to whether femoral venous pressures (FVP) are comparable to IAP measured with an intra-vesical technique. Current recommendations by the WSACS do not recommend FVP as a surrogate for IAP.[7,8] The controversy surrounds the fact that it is often not clear as to what pressure the femoral catheter is measuring in experimental reports, increasing the confusion surrounding the technique. In some studies, the catheter is intra-abdominal, indicating the pressure obtained is from the IVC.[183] Others report that the pressure is from the extra-abdominal vessel, based on the position of the catheter tip.[136] Additional studies report the use of femoral venous catheters advanced into the IVC within the thoracic cavity and that these catheters are also used to obtain central venous pressure (CVP).[176] Results are conflicting as to the significance and relevance of correlations between FVP and IAP.

In a study performed in anesthetized swine in dorsal recumbency, blood flow as well as FVP were determined in the left femoral vein, located in the groin and upper region of the thigh and compared to bladder pressure and pressures obtained from the IVC.[136] In this model of IAH using fluid distention, blood flow in both the IVC and FVP showed a stepwise increase as pressure in the abdomen rose to 40 mmHg. Pressures in the IVC and femoral vein showed stepwise increases along with increases in intra-

vesicular pressure increased. As pressure continued to increase to 40 mmHg and then returned to 15 mmHg, measurement of IAP in all three instruments were not significantly different (except for one timepoint, initially at 15 mmHg). Heart rate and mean arterial blood pressure were not affected in these swine. CVP (measured by inferior vena cava pressure) was also elevated as IAP increased, and it was stated that CVP corresponded well with intra-vesicular pressure and pressure in the IVC, although values were not provided. However, in a second study measuring IAP in swine, IVC showed poor agreement with direct pressure measured of the abdomen, with a correlation of 0.9, bias of 1.81, and precision of 4.47 mmHg.[128]

Decreases in femoral venous blood flow with elevations in IAP have also been shown in other experiments in animal models.[70,71,136,184] In Barnes' canine study, infusion of Tyrode's solution into the abdomen produced an increase in FVP that highly correlated with pressure in the abdomen ($r^2=0.98$), but the raw measurements were only 90% of that seen in the abdomen.[70] In Harman et. al.'s study performed in dogs, inferior vena cava pressure (IVCP) equaled IAP measured directly from the peritoneum at pressure up to 40 mmHg.[93] In rabbits, pressure in the IVC correlated well with bladder pressures, whereas gastric, rectal, superior vena cava pressure and brachial pressures did not.[133] Gudmondsson et. al. also noted a good correlation between IVCP, bladder and FVP in swine.[136] In a study in dogs insufflated with CO_2, saphenous venous pressure was observed to be reliable indicator of insufflation, and rapidly noted a loss of IAP when monitored in a continuous fashion.[185] In contrast, others noted less success in using FVP for IAP measurement in animal models. In a number of studies, a positive correlation

could not be shown, and FVP or IVCP often overestimated IAP in swine and dogs.[92,134,184]

One reason for the complications in validation of FVP is the standard IAP used for comparison of methods. Regli et. al. noted a good correlation with IAP and FVP in a porcine model of IAH under anesthesia.[183] Although a femoral venous catheter was used, it was confirmed to have a 15 cm intra-abdominal length, indicating that it was actually IVCP that was obtained. Results found that the pressure in the abdomen measured by an intra-vesicular catheter was strongly correlated with FVP/IVCP (r^2: 0.89; bias: 5 mmHg; precision: 3.8 mmHg). Validation against an indirect method of measurement may have introduced bias, and these results may have been partially influenced by the fact that the bladder pressure in this study was obtained as a mean, rather than at end-expiration. Positive bias was also found in other studies that reported a higher FVP when compared to intra-vesicular pressures.[136]

In humans, a preliminary study in patients undergoing laparoscopic surgery found that FVP significantly increased in tandem with step-wise increases in intra-abdominal insufflation using carbon dioxide gas.[186] However, most studies in humans also employ intra-vesicular pressures as the standard, therefore reports of successful validation of FVP have been variable. Markou et. al. noted a good correlation in mechanically ventilated patients between bladder pressures and FVP at pressures above 15 mmHg.[187] Good correlation was also observed in patients undergoing renal replacement therapy between intra-vesicular pressures and a device that measured pressure from the IVC (r^2=0.998).[188]

A recent multi-institutional study also noted good correlation in humans, but only when IAP was greater than 20 mmHg (bias: 0.7 mmHg; LOA: -3, 4.6 mmHg).[189]   A receiver operator curve demonstrated an area under the curve of 0.83, and with a cut off of 11.5 mmHg provided a sensitivity for IAH of 84.8% and 67% specificity, allowing for prediction of IAH in clinical patients. For prediction of ACS, the threshold was determined to be 14.5 mmHg. Part of the variability in this study may be attributed to the variations in technique at the participating institutions.

Howard et. al. obtained similar results in a study conducted in ventilated and sedated humans.[190]   Correlation was good between the two methods ($r^2 = 0.8$), but the confidence intervals were again wide (0.5 to 2.0 mmHg) indicating that FVP were not adequate to predict accurate measures for IAP (bias: 3.2 mmHg, LOA -4.1, 10.4 mmHg). However, the ROC curve noted an area under the curve of 0.87 with a cutoff of 12 mmHg (95% CI of 0.74-0.94) which provides evidence that support the use of FVP for prediction of IAH. Again, comparisons of indirect methods of IAP measurement likely confounds both precision and bias in the results of these studies.

The advantage of FVP, despite its limitations, is the ability to continuously monitor IAP without more invasive methods using nasal or bladder catheters. The drawback is that it is still an indirect method of analysis. Based on findings in humans, FVP monitoring can be used to continuously monitor patients with grade 3 and 4 IAH, for the development of ACS.[189]

1.7.5   Intra-Abdominal Volume Measurement

Intra-abdominal volume (IAV) has been estimated in humans using anthropomorphic indices and imaging techniques including computed tomography and magnetic resonance imaging. While body mass index has an anthropomorphic index formula to estimate IAV, it does not correlate with compliance and only correlates well with IAP at resting volumes in healthy individuals.[23] Changes in circumference may be a better measure for IAP, and a number of different ratios have been used similarly to calculate IAV from external dimensions.[121]. A novel method for calculating IAV using three-dimensional ultrasound, magnetic resonance imaging, and computed tomography may be promising a tool in the future.[35,36,191,192] However, the size of large animal patients may limit the usefulness of this technique with the technology currently available.

1.7.6   Abdominal Compliance Measurement

External palpation is often performed to examine intra-abdominal tension, as well as tension in the abdominal wall musculature.[193] This method cannot quantify abdominal compliance, and has not been validated to do so.[193]   Compliance has been assessed by determination of IAP after addition or removal of fluid from the abdomen.[39,40,194] It has also been estimated by analysis of changes in IAP caused by mechanical ventilation. Using a low flow, pressure-volume loop, compliance is calculated by the change in tidal volume divided by the change in mean IAP at the start and end of the pressure volume loops. Large respiratory excursions on a continuous IAP tracing indicate a lower abdominal compliance.[195] Compliance can also be estimated from the abdominal pressure

variation, where the change in IAP is inversely proportional to the compliance, to provide a continuous non-invasive estimate of compliance.[39]

Measurement of abdominal compliance may help in prediction of complications that may occur with increased IAP, including the development of ACS. Risk factors for IAH associated with increased compliance include anthropomorphic measurements, and those related to abdominal wall or diaphragmatic disease or disorders. (Table 1-3) However, changes may also be observed that increase abdominal wall compliance, including pregnancy, and slowly developing abdominal tumors. (Table 1-4) In these patients, a high IAP may be less predictive for the development of IAH, and must be taken into consideration when evaluating a single observation of IAP.[33,40]

1.8   Consensus Guidelines for Equine Intra-Abdominal Pressure Measurement

Currently, there is no consensus available to provide guidelines for normal ranges of IAP in any animal species, including the horse, as well as the gradations of IAH that may guide therapeutic intervention. An arbitrary guideline was proposed by one author in dogs, where a pressure of 5-10 cm H_2O was normal, 10-20 cm H_2O was mild IAH, 20-35 cm H_2O was moderate to severe IAH, and pressures over 30 cm H_2O indicated that intervention was indicated.[196] Consensus statements for IAP measurement in horse will require standardizations of methods, as well as the development of a method for IAP that can be readily accepted by the veterinary community in regards to ease of use, accessibility, cost, and effect on outcomes.

Table 1-1 Grades of intra-abdominal hypertension as defined by the World Society of the Abdominal Compartment Syndrome. **Adapted from Malbrain ML, Cheatham ML, Kirkpatrick A, et al.** Results from the International Conference of Experts on Intra-Abdominal Hypertension and Abdominal Compartment Syndrome. I. Definitions. *Intensive Care Med* 2006;32:1722-1732.

Intra-abdominal Hypertension Grade	Intra-abdominal pressure
Grade I	12-15 mmHg
Grade II	16-20 mmHg
Grade III	21-25 mmHg
Grade IV	>26 mmHg

Table 1-2 Risk factors for the development of intra-abdominal hypertension and abdominal compartment syndrome in humans. **Adapted from Malbrain, M.L., Cheatham, M.L., Kirkpatrick, et. al.** Results from the International Conference of Experts on Intra-Abdominal Hypertension and Abdominal Compartment Syndrome. I. Definitions. *Intensive care medicine* 2006;32:1722-1732.

Risk factor	Examples
Decreased abdominal wall compliance	• Abdominal surgery • Major trauma • Major burns • Prone positioning
Increased intra-luminal contents	• Gastroparesis/gastrointestinal distention • Ileus • Colonic pseudo-obstruction • Volvulus
Increased intra-abdominal contents	• Pancreatitis • Distended abdomen • Hemoperitoneum/pneumoperitoneum • Peritonitis/ascites • Intra-abdominal or retroperitoneal neoplasia • Laparoscopy with excessive insufflation • Liver dysfunction/cirrhosis • Peritoneal dialysis
Capillary leak or fluid resuscitation	• Acidosis • Damage control laparotomy • Hypothermia • Massive fluid resuscitation/positive fluid balance • Polytransfusion
Miscellaneous	• Age • Bacteremia • Coagulopathy • Body position • Incisional hernia/repair • Mechanical ventilation • Obesity • Positive end-expiratory pressure >10 mmHg • Pneumonia • Sepsis • Shock/hypotension

Table 1-3 Categorical risk factors for decreased abdominal wall compliance in humans. **Adapted from Malbrain, M.L., Roberts, D.J., De Laet, I., et. al.** The role of abdominal compliance, the neglected parameter in critically ill patients - a consensus review. Part 1: definitions and pathophysiology. *Anaesthesiol Intensive Ther* 2014;46:392-405.

Categorical Risk Factor	Examples of Risk factor
Signalment	• Body shape (apple) • Visceral adiposity • Height (short) • Sex (male) • Age (young, increased elastic recoil) • Obesity
Comorbidities or increased non-compressible intra-abdominal volume	• Fluid overload • Abdominal fluid collections • Sepsis, burns, trauma • Coagulopathies • Ileus (fluid stasis) • Hepatomegaly or splenomegaly
Abdominal wall or Diaphragmatic disease or conditions	• Interstitial edema • Burn eschars • Tight abdominal wall closure • Abdominal bandages • Body positioning • Hernia repair • Pain/muscle contraction • Muscle development • Pneumoperitoneum • Abdominal wall hemorrhage/hematoma • Mechanical ventilation/PEEP • Chronic recurrent airway obstruction/emphysema • Pleuropneumonia

Table 1-4 Categorical risk factors for increased abdominal wall compliance in humans. **Adapted from Malbrain, M.L., Roberts, D.J., De Laet, I., et. al.** The role of abdominal compliance, the neglected parameter in critically ill patients - a consensus review. Part 1: definitions and pathophysiology. *Anaesthesiol Intensive Ther* 2014;46:392-405.

Categorical Risk Factor	Examples of Risk factor
Signalment	• Body shape (ellipse, pear) • Peripheral adiposity • Height (short) • Sex (female) • Age (geriatric loss of recoil) • Lean or normal BMI
Comorbidities or increased non-compressible intra-abdominal volume	• Absence of fluid overload • Normothermia, normal pH, lack of coagulopathies • Tympany
Abdominal wall or Diaphragmatic disease or conditions	• Previous pregnancy • Previous laparoscopy or abdominal surgery • Weight loss • Chronic IAH • Umbilical or body wall hernia • Escharotomy • Open abdomen or temporary abdominal closure • Sedation/analgesia/muscle relaxation • Lung protective ventilation

Figure 1-1 Abdominal pressure-volume curve demonstrating effect of compliance on intra-abdominal pressure. **Adapted from Malbrain ML, Peeters Y, Wise R.** The neglected role of abdominal compliance in organ-organ interactions. *Crit Care* 2016;20:67. An abdominal wall with low compliance is shown with the upper curve (squares), and normal compliance is represented by the lower curve (circles). A small increase in the abdominal volume in a normal animal, e.g. from 4 to 8 liters, will produce a small increase in the IAP, whereas the same increase in volume in an abdomen with low compliance will lead to a larger increase in the IAP. At an abdominal volume of 8 liters, the low compliance curve has already reached the critical point where any change in volume will dramatically increase the pressure in the abdominal cavity.
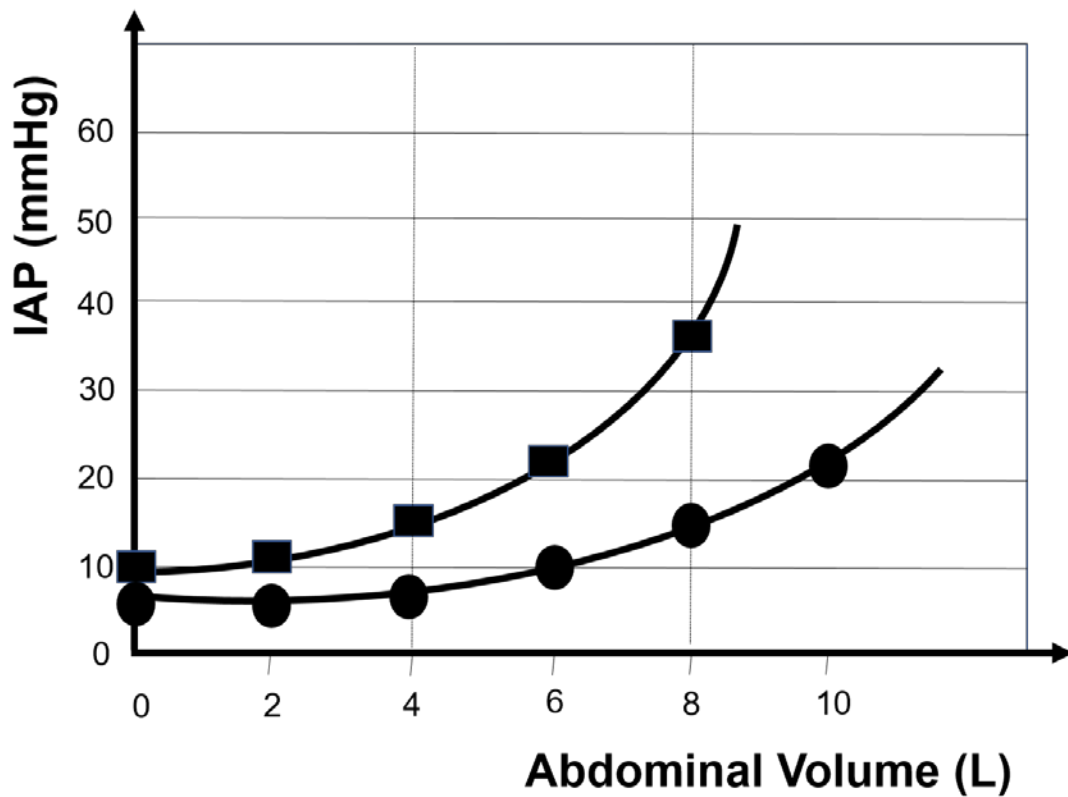
Figure 1-2 Relationship between intra-abdominal pressure, intra-abdominal volume and abdominal wall compliance. **Adapted from De Keulenaer BL, De Waele JJ, Powell B, et al.** What is normal intra-abdominal pressure and how is it affected by positioning, body mass and positive end-expiratory pressure? *Intensive Care Med* 2009;35:969-976. Intra-abdominal volume is compared against intra-abdominal pressure. The arrows represent the direction of movement associated with the action of the rib cage inspiratory muscles, the abdominal wall musculature, and the diaphragm. The movement of the diaphragm is also dependent on abdominal compliance, but its vector remains within the sector between abdominal compliance (Cab) of 0 and infinity. The multiheaded arrow represent the directional force of gravity. Uniform compression by the muscular contractions results in a homogenous change in pressure. Shear forces are not included, and add spatially diverse pressure gradients.
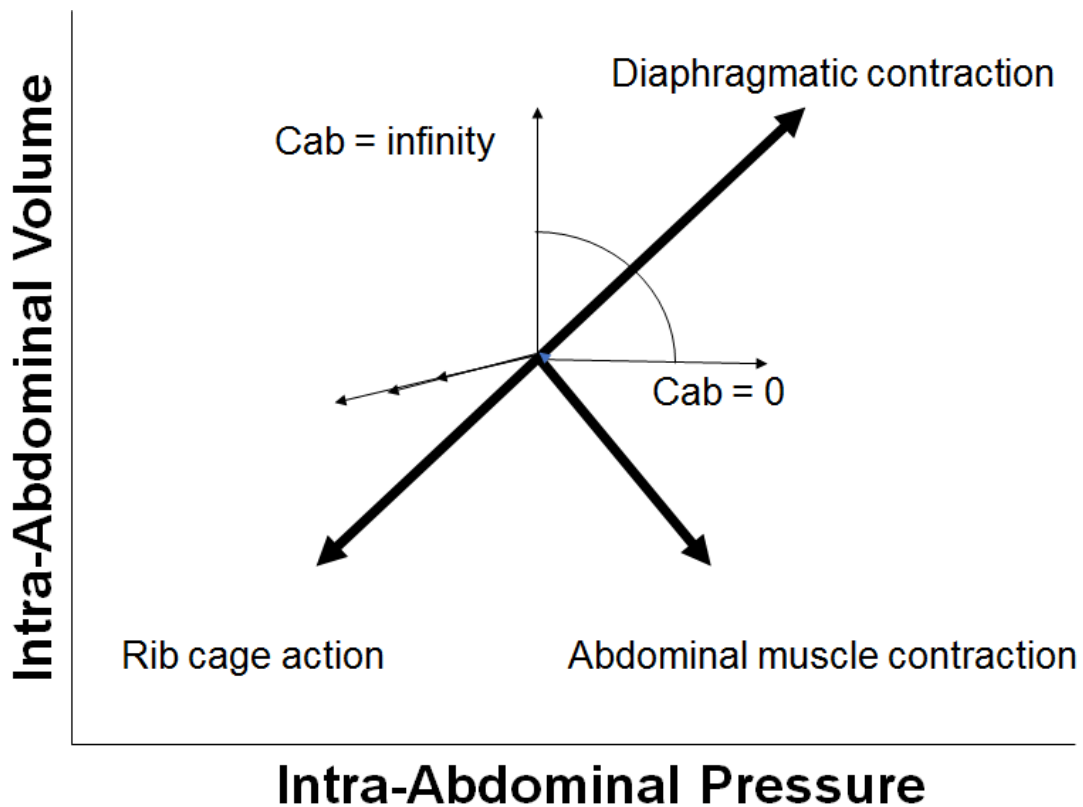
Chapter 2

Abstract

Intra-abdominal pressure measurement is a method used to determine the pressure within the abdomen to clinically assess its effects on tissue perfusion in critically ill patients. Although direct pressure measurement from the abdominal cavity is more accurate, it is also invasive. Therefore, indirect pressure measurement using a bladder catheter is preferred in other species. In horses, the method for direct intra-abdominal pressure measurement has not been standardized, and critical evaluation of indirect methods have not been performed to validate these techniques. The purpose of this study was to develop a direct method for measurement of intra-abdominal pressure in the standing horse to create a reference interval for abdominal pressure in normal horses. The second purpose was to compare these pressures to pressures obtained from the urinary bladder, to determine the correlation between techniques, and to identify the optimal infusion volume needed to obtain an accurate indirect measure of intra-abdominal pressure. The study used ten healthy adult horses; five male and five female. Direct intra-abdominal pressures were obtained through an intra-peritoneal cannula, referenced to the midpoint between the tuber ishii and the point of the shoulder. The

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indirect measurements of intra-abdominal pressure were performed using a urinary catheter, with a reference point at the level of the tuber ishii. The intra-abdominal pressures obtained directly from the peritoneal space were found to be subatmospheric and were shown to decrease as the horse's weight increased. Head position did not influence pressure measurements obtained. The averaged baseline indirect pressure measurements from the bladder were significantly different from the pressure measured directly from the abdominal cavity (P<0.001). The indirect pressures measured from the bladder were altered by the volume of fluid infused into the bladder, and were statistically increased from baseline with an instillation volume of 100 ml (P=0.004). Correlation between the direct and indirect methods of measurement was low to moderate (Pearson's correlation coefficient: -0.38 to 0.58). The results of this study show that indirect pressure measurement of intra-abdominal pressure using a urinary catheter in standing horses is not correlated with pressures measured directly from the abdominal cavity. These methods of measurement cannot be used interchangeably.

Keywords   equine, bladder pressure, intra-abdominal pressure, intra-peritoneal pressure, intra-vesicular pressure

2.1   Introduction

A pathological increase in pressure in the abdomen is described as intra-abdominal hypertension (IAH) in humans, and is defined by sustained or repeated measures of intra-abdominal pressure (IAP) greater than 12mmHg.[8] With increased IAP, venous return and cardiac output decrease, and tissue perfusion is reduced.[8,197] IAH is an independent risk factor for multiple organ dysfunction, and organ failure resulting from IAH is known as abdominal compartment syndrome (ACS).[8,47]

Three causes of IAH in human medicine include decreased body wall compliance (e.g. tight abdominal closures), increased intra-abdominal contents (e.g. due to ileus, ascites, or hemoabdomen), as well as intra-abdominal hypertension secondary to large volume resuscitation resulting in capillary leak syndrome, reperfusion injury, and cytokine release.[197-199] Similar risk factors for IAH are present in equine patients, and recognition of IAH in this species would allow for development of therapies to prevent multiorgan dysfunction from compartment syndrome.

IAP can be obtained directly, through an intra-peritoneal catheter, or through indirect methods, using catheters introduced into one of a number of intra-abdominal organs that transfer pressure through their walls.[113] The indirect method most commonly implemented in human patients involves infusing a small volume of fluid into the bladder through a transurethral catheter to obtain a solid fluid column to the bladder, followed by connection of this continuous fluid column to a pressure transducer or water manometer to obtain the pressure.[8,113,125] This indirect method using intra-vesicular pressures has been investigated as technique for IAP measurement in veterinary patients,[200,201] as well as in canine and porcine models of human IAH.[22,123,136]

The relevance of work performed in other species, and its application to the horse, is difficult to assess based on anatomical differences that may alter transmission of pressures in the peritoneal cavity. In the equine patient, external variables such as head position, body size, abdominal compartmentalization, urethral length, temperament, and muscle tone all may influence IAP measurements. Recent research models measuring IAP in horses have used both a direct and indirect method.[148,159] However, a standard technique for the horse has not been defined, and many of the variables that may affect the measurements obtained, including the effects of the abdominal viscera and the height of catheter placement, still require investigation.

The purpose of this study was to describe a method for direct IAP measurement, and to obtain measurements using this technique in normal, standing horses. Our objectives were to (1) assess the effect of variables including head position and body weight on direct pressure measurements and (2) compare this direct method to an indirect method of pressure measurement using a transurethral catheter. Given that the volume infused into the bladder to measure indirect pressures in humans affects the pressures obtained,[136,137,139,152,202,203] the final objective was (3) to determine the optimal volume for indirect IAP measurement to further improve this technique. The hypothesis was that measurements obtained by both methods would be repeatable within each horse and between horses, and that significant correlation between the techniques would allow either method to be used for measurement of IAP in the horse.

2.2   Materials and Methods

2.2.1   Animal Selection

All procedures were approved by Auburn University's Institutional Animal Care and Use Committee (Protocol #2008-1424). Ten healthy adult horses, 5 geldings and 5 mares, weighing an average of 486 kg (range, 362–537 kg) were used for this investigation. Breeds represented included 3 Thoroughbreds, 2 American Quarter Horses, 2 mixed breeds, 1 Arabian, 1 Paint, and 1 Tennessee Walking Horse. Ages ranged from 2 to 22 years. Each horse was fasted for 24 hours before initiation of the procedures, with water being withheld the last 3 hours. Fasting was instituted to reduce the effects of acute gastric fill on the pressure measurements.

2.2.2   Instrumentation

On the day of the procedures, the horses were weighed, restrained in stocks, and sedated with detomidine hydrochloride at a dose of 0.01 mg/kg, intravenously (Pfizer Animal Health, Exton, PA). For direct IAP measurement, a modified abdominocentesis procedure was performed. The height of the midpoint of the tuber ishii and the point of the shoulder (cranial eminence of the greater tubercle of the humerus) were measured and recorded. A point midway between these measurements was calculated as the height for placement of the peritoneal cannula. (Figure 2-1) The final site was chosen at that height, approximately 12 cm caudal to the last rib in the right flank, and the area was clipped and aseptically prepared. Lidocaine (2%, 0.1mg/ kg; Hospira, Inc., Lake Forest, IL) was injected into the subcutaneous tissue and muscle at the selected site, and a stab incision was made through the skin using a number 15 scalpel blade. An 8-cm metal teat cannula was then bluntly introduced into the abdomen. (Figure 2-2) Before placement, a 15-cm

extension set (Macrobore extension set no. 19328-48, Hospira, Inc., Lake Forest, IL) with an injection port near the hub was attached to the teat cannula and clamped to prevent introduction of air into the abdomen when the cannula was introduced. Sterile, water-based lubricant (Surgilube, E. Fougera and Co., Melville, NY) was also applied after cannula introduction at the site of entry to reduce the risk of air entering the abdomen.

For direct measurement of IAP, a 20-gauge needle attached to the tubing from a water manometer (CVP Manometer, Mila International, Inc., Erlanger, KY) was introduced into the proximal injection port of the extension set, into the cannula lumen. (Figure 2-2) A fluid column was established from the water manometer using balanced electrolyte solution (Veterinary Normosol R, Abbott Laboratories, North Chicago, IL), and the 3-way stopcock was opened on the water manometer to measure the intra-abdominal pressures. The manometer was zeroed at the height measured for introduction of the cannula.

For indirect IAP measurement, the open-system technique used by Kron was applied,[22] with a modification to allow for introduction of the priming volume after connection to the water manometer. (Figure 2-3) The vulvae in the mares and the urethral processes in the geldings were cleaned and prepared. A catheter was placed in the bladder of each horse, using a stallion catheter (Stallion urinary catheter, Jorgensen Laboratories, Inc., Loveland, CO) for the males, and a 24 French Foley catheter for the females, and the urinary catheters were connected to the water manometer by pressure tubing (Pressure monitoring extension tubing, Mila International, Erlanger KY) and a 3-way stopcock. (Figure 2-2) Balanced electrolyte solution, at body temperature (37ºC), was introduced using a 30-mL syringe attached to this stopcock. The fluid reservoir was linked by a fluid

administration set to the 3-way stopcock attached to the manometer. For pressure measurement, the bladder was emptied of urine, and then the manometer tubing was attached. Entrainment of air into the system was prevented by clamping the urinary catheter before connection to the manometer. The desired amount of fluid plus an amount to fill dead space present in the urinary catheter (17 mL) was infused into the bladder. The stopcocks were then opened to the water manometer to obtain a pressure reading with the manometer zeroed at the tuber ishii.

2.2.3   Intra-Abdominal Pressure Measurement

After instrumentation, the measurement of IAP began approximately 30 minutes after sedation was administered. To assess baseline direct IAP, the effect of body weight, and the effect of varying horse's head position, pressures were measured with the head elevated above, at, and below the withers. The measurements were repeated 3 times at each head height. For comparison of direct and indirect IAP, measurements were obtained simultaneously for 4 separate volumes of fluid (0, 50, 100, and 200 mL), which were introduced into the bladder in random order. For each set of measurements at each volume, the bladder was first evacuated, and the desired volume was infused slowly into the bladder. Measurements were obtained after the bladder had equilibrated for 1 minute, based on human studies,[139,152,202] to reduce the effect of detrusor muscle contraction. The abdominal catheter was flushed at the time of fluid instillation into the bladder, and was also allowed to equilibrate for 1 minute. A total of 9 measurements were obtained for each intra-vesicular volume over 5 minutes. According to previous work, each

measurement was obtained when IAP was the lowest and when abdominal contractions were absent,[8,114] noted in these horses to be at the end of the inspiratory phase.

2.2.4   Statistical Analysis

The averaged direct IAP obtained from the abdomen was calculated for each horse with the head in a neutral position to give a baseline pressure with no external effects. The correlation of body weight to mean baseline IAP was determined through a Pearson's product moment correlation coefficient and regression analysis. All direct IAP measurements were normally distributed; therefore, a single factor, repeated measures ANOVA was performed to determine the effect of the head position on pressure. Post-hoc analysis using a Newman-Keuls multiple comparison post-test was used to determine significance, when indicated. The simultaneous indirect and direct IAP measurements were normally distributed, and were evaluated using a 3-way ANOVA, followed by post-hoc analysis of least squares mean to assess the effects of the instillation volume. If statistical significance was met, it was confirmed using a Bonferroni's correction of the P value. Correlation of the 2 methods of pressure measurement was assessed using a Pearson's correlation and regression analysis. All correlations were further assessed with analysis of difference in fits and Cook's distance parameters to assess extreme values for undue influence. Where indicated, results are reported as mean (standard deviation), with a 95% confidence interval (CI). All statistical analyses were performed by commercially available statistical software packages (SAS Analytics Pro, SAS Institute Inc., Cary, NC; GraphPad Prism 5 for Windows, GraphPad Software Inc., La Jolla, CA; Minitab 15

Statistical Software, Minitab Inc., State College, PA). Statistical significance was set at $P < 0.05$.

2.3 Results

The averaged direct IAP measured from standing, normal horses with the head in a neutral position was -1.80 cm H_2O (1.61 cm H_2O; 95% CI: -2.80 to -0.80 cm H_2O), ranging from -5.0 to 0.3 cm H_2O (median: -1.65 cm H_2O). Serial measurement of direct IAP was performed in each horse, which obtained consistent readings (variance 0.00 to 0.85, average standard deviation: 0.22). A significant relationship was found between horse's weight and direct IAP measurements, which were negatively correlated (Pearson's r: -0.67, r^2: 44.5%, standard error: 1.28, $P = 0.04$). (Figure 2-4) Perfect correlation would be evident if Pearson's r approached 1 or -1. The slope of the regression line (-0.02) indicates a decrease in direct IAP of 2 cm H_2O for every 100 kg increase in body weight. Head position (up, down, or neutral) had no effect on pressure, based on a single factor, repeated measures ANOVA ($P = 0.15$). Of the 3 positions, the neutral position had the least variation in pressure. A Newman Keuls multiple comparison post-test confirmed the significant lack of difference between the means ($P > 0.05$) for all comparisons of head position.

The averaged indirect IAP of -8.63 cm H_2O (4.37 cm H_2O; 95% CI: -13.05 to -4.21 cm H_2O) measured with 0 mL of fluid infused into the bladder was more negative than the direct pressure and ranged from -1.98 to -14.06 cm H_2O (median: -7.84 cm H_2O). (Table 2-1) Averaged direct IAP measured simultaneously was -0.11 cm H_2O (1.36 cm H_2O; 95% CI: -0.92 to 0.7). Measurements of direct and indirect IAP were

repeatable within each horse for all volumes infused into the bladder; however, the variance of indirect pressures between horses was 3-10 times the variance of direct pressure measurement.

Indirect measurements of IAP were not obtained by the manometer in 4 horses (2 males, 2 females), noted by a lack of respiratory variation or oscillations in the fluid column with abdominal ballottement, for the bladder instillation volume of 0 mL. The indirect and the corresponding direct IAP measurements for this volume in these horses could not be compared. Once fluid was infused into the bladder at higher volumes ($\geq$50 mL), there were no additional problems noted with indirect pressure measurement using the manometer.

The effect of variations in intra-vesicular fluid volumes on indirect and direct measurement of IAP was assessed by a 3-factor ANOVA and post-hoc analysis of least squares mean, comparing the horse, volume infused, and method of pressure measurement. (Figure 2-5) While direct measurement of IAP was not altered with increasing volumes of fluid infused into the bladder as compared with the baseline of 0 mL ($P>0.58$ for all comparisons), indirect pressures increased from baseline as the volume infused in the bladder increased. Comparisons of the indirect IAP at each instillation volume noted a statistically significant increase in indirect IAP when 100 mL of fluid was infused in the bladder when compared with pressures measured when 0 mL was infused ($P=0.004$). The difference between pressures measured with 100 mL and 0 mL instillation volumes was also significant when a Bonferroni's correction factor for multiple comparisons was applied ($P=0.008$).

The direct IAP were significantly different from the indirect pressures for all bladder volume comparisons, based on the 3-factor ANOVA and post-hoc analysis of least squares mean (P<0.001). The regression analyses were graphed for each correlation, and a suspected outlier (50 mL instillation volume, direct pressure: -5.47 cm H_2O, indirect pressure: -8.19 cm H_2O) was noted. Based on a significant Cook's distance measure of 1.85 (>1 is suspicious), and a difference in fits of 1.85 (significant influence for this comparison $\geq$1.309), Pearson's correlation and regression analysis were reassessed without this point. Pearson's r value improved from 0.12 to 0.38, and the r^2 value increased from 1.5% to 14.7%. This point was determined to be an outlier and was removed from the final analysis. No additional points were deleted. Pearson's correlations show a low to moderate positive correlation between the pressures measured by the intra-vesicular catheter and those measured directly in the abdomen, with values for Pearson's r ranging from 0.38 to 0.58. (Table 2-2 and Figure 2-6) None of the correlations reached statistical significance.

2.4   Discussion

In this study, the IAP obtained directly with an intra-peritoneal cannula and indirectly using an intra-vesicular catheter were subatmospheric. These pressures were consistent and repeatable within each horse, but showed increased variation between horses for the indirect method. As the weight of the horse increased, the direct IAP decreased significantly. Although the position of the head did not have a statistical effect on pressure, variation in pressure measurement was lowest with the head in a neutral position.

The fluid volume infused into the bladder influenced the indirect pressures obtained, indicated by an increase in the pressures measured as the volume infused increased. At an infusion volume of 100 mL, the difference was statistically significant. Correlation between the techniques was not statistically significant, indicating that the 2 techniques cannot be used interchangeably to measure normal IAP, and that individual reference intervals must be developed for both techniques.

The cannula site for direct pressure measurement was selected based on 3 factors, since a standard site is not defined for horses. First, the site was extrapolated from the mid-axillary line, the recommended reference point for indirect pressure measurement in humans.[8] This site was easily accessible in the flank, and used 2 boney landmarks to allow for consistent selection of an entry point. Second, this site was chosen to allow for a more accurate measurement of IAH. Previous work has shown that the mass of the abdominal contents above the site of measurement influences the IAP measured.[113,114,197,204] Because the majority of gastrointestinal viscera lies beneath the cannula, the site selected should reduce error due to normal variations in abdominal fill, but still allow space above the cannula to measure any abnormal increase in IAP in future studies. Finally, the catheter site was selected to allow for a more relevant comparison between direct and indirect IAP measurement by intra-vesicular and intra-gastric catheters. Based on the unknown effects of the abdominal viscera on the pressures measured at the various positions of the catheters in the abdomen, the site was selected to approximate a similar height in the abdomen for all 3 catheters in an effort to reduce this effect.

To allow for a direct comparison with previous reports, indirect IAP measurements in this study were zeroed at the tuber ishii. Assuming similar external variables, the pressures obtained in this study appear to show agreement with previous data obtained from standing horses.[148,159] In these reports, indirect pressures range from subatmospheric to slightly positive when zeroed at the pubis (95% CI: -7.9 to 2.3 cm H_2O),[159] and up to 7 cm H_2O when zeroed at the tuber ishii.[148] These studies used a 100 mL bladder infusion volume to obtain pressures, and at that volume we obtained a mean of -7.43 cm H_2O (4.74 cm H_2O; 95% CI: -10.37 to -4.49 cm H_2O). Our more negative range of pressures could be attributed to subtle variations in determination 0 point, due to the size and shape of the tuber ishii used as reference. In addition, the study that zeroed to the pubis would also have produced values slightly more positive than studies referenced to the tuber ishii, due to its more ventral reference point relative to the abdominal contents.

In our study, direct IAP were subatmospheric, which is expected to allow for normal venous return and perfusion of the abdominal organs.[8,103,197] However, direct pressures measured previously through a ventral midline cannula in horses were extremely positive (95% CI: 17.9–43.1 cm H_2O).[159] The most likely explanation for the higher IAP described previously is the increased weight and volume of viscera above the cannula when pressures were measured on ventral midline. Higher direct pressures are noted in other species, as pressures are measured more ventrally related to the known effects of gravity, the mass and deformability of organs, and compressive external forces.[113,114] It is unknown if these forces may inhibit the ability to detect alterations in

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normal IAP in the horse. Future studies would be indicated to compare these effects on direct IAP at different sites of cannula placement and at increased IAP.

The effect of body weight on direct IAP was evaluated due to the significant positive effect of body mass index on pressure measurement in humans.[2,18,205,206] In horses, body weight correlates well with body mass index, allowing weight to substitute for body mass index for this comparison.[207] In this study, direct pressures measured were negatively correlated to body weight. An explanation for this finding could relate to either variations in body condition, or a relative increase in abdominal dimensions compared with body size as body weight decreased. Future studies using this direct technique may require assessment of abdominal girth and body condition scores to assess the correlation. Although a decrease of 2 cm H_2O for each 100 kg increase in weight may not be clinically relevant in adult horses, it may significantly alter pressures measured in smaller equids.

The direct IAP measured in our study were not significantly affected by the head height. It is known that indirect blood pressure measurement can vary with head position; raising horse's head can falsely increase blood pressure, and conversely, the pressure decreases if the head is lowered.[208] This may be relevant when applying goal-directed therapy endpoints because abdominal perfusion pressure, calculated as the mean arterial blood pressure minus the IAP, has been shown to be a better resuscitation endpoint and predictor of outcome for critical human patients than IAP, mean arterial pressure, pH, base deficit, lactate, or hourly urine output.[29] Although head position did not affect IAP in our study, consistent head position (ideally in the neutral position) is likely necessary for accurate calculation of abdominal perfusion pressure.

Previous assessments of indirect IAP measured with an intra-vesicular catheter in the horse have not evaluated the effect of infused fluid volume on bladder pressures. Our results indicate that indirect pressures were increased with increasing bladder infusion volumes. We speculate that this may be due to increased detrusor tone as has been reported in the human literature.[136,137,139,152,202,203] Current recommendations suggest that bias can be reduced by infusing a minimal amount of fluid, <25mL in humans, to establish a column of fluid for a pressure reading.[8,131,137,139,203] The results of this study show no effect on pressures with infusion volumes of 50 mL or less, and more consistent readings obtained with volumes > 0 mL. Therefore, the 50-mL infusion volume appears to allow for the most dependable indirect pressure measurement in the horses in our study.

When indirect IAP were compared with direct pressures across the range of bladder volumes in this study, the correlations were only low to moderate for all volumes infused. These correlations improved slightly as volume increased, but were not significant. Human studies that examined the correlation of direct and indirect IAP across a range of volumes showed much higher correlation coefficients for all volumes, with Pearson's r ranging from 0.78 to 0.97.[131,152] Anatomical differences, including the position of the bladder (retroperitoneal) in the human versus the horse (intra-peritoneal), make direct comparisons between studies difficult.[136] However, it would be reasonable to assume that the correlation must be better if methods of measurement are to be compared or used interchangeably. The lack of correlation in this study may be related to the large variation in indirect IAP noted between horses, which could affect the establishment of a standard for documentation of IAH by this indirect method. Further investigation will be

required to determine if these findings hold true in a larger population and across a range of IAP.

Critique of our methods of direct and indirect IAP measurement could be based on use of a water manometer, the under- and over-damping inherent in manometer tubing, or from error introduced by air bubbles often present in these systems.[113] In addition, variation between measurements may have resulted from detrusor muscle tone directly increasing bladder pressures due to temperature of the infused fluid or filling rate, among others.[137,202] Contraction or relaxation of the abdominal wall due to movement or level of sedation may also have altered the pressures obtained, and the vertical heights of the catheters for each method were not identical, which may have allowed the weight of the viscera to affect the measurements. It is difficult to assess each issue separately, and each confounding factor could affect either method of intra-abdominal pressure measurement in a different way.

Indirect IAP measurement with an intra-vesicular catheter has been reported to be the gold standard in humans.[8] However, numerous authors have called this method into question based on variability in technique, inherent bladder tension, reference level, body position, and indirectness.[113,131,135,136] Based on the results of this study and our techniques, we have shown that indirect IAP were repeatable within each horse, but not between horses, and that indirect pressures did not significantly correlate to direct pressure measurements. Because of the large variation in measurement of indirect pressures between horses, validation of this technique may be difficult. Advantages of this technique include cost effectiveness and simplicity of measurement, which would make measurement of indirect IAP an effective screening tool for trends in pressure.

Drawbacks to this method include possible iatrogenic urinary tract infection, and problems caused by active urination by the horse, which tended to dislodge the catheters in our subjects, and could make repeated measurement difficult.

Potentially serious complications of direct IAP measurement, using the technique described in this study, include enterocentesis, peritonitis, pneumoperitoneum, and local subcutaneous infection or abscess formation. The risks of these complications could increase in horses with increased IAP or visceral distention, as well as the risk of cannula occlusion by the viscera. However, this method is comparable in risk to a teat cannula abdominocentesis, commonly performed in horses with abdominal disease. Clinical use of this procedure may be contraindicated in any horse in which cannula abdominocentesis was deferred, or in horses where long-term IAP monitoring is required. Refinement of this method for direct pressure measurement would allow for evaluation of additional, less invasive, indirect methods of IAP measurement in the research setting. Therefore, validation of this technique at higher IAP is warranted.

Table 2-1 Summary of indirect and direct intra-abdominal pressure measurements averaged between all horses by bladder infusion volume, with variation assessed by standard deviation and variance. **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.

Bladder instillation volume (mL)	Mean pressure (cm H_2O)		Standard deviation (cm H_2O)		Variance (cm H_2O)		95% Confidence interval	
	Indirect	Direct	Indirect	Direct	Indirect	Direct	Indirect	Direct
0	−8.63	−0.11	4.37	1.36	19.07	1.84	± 4.42	± 0.81
50	−7.85	−0.83	3.70	1.89	13.68	3.59	± 2.29	± 1.17
100	−7.43	−0.29	4.74	2.12	22.45	4.44	± 2.94	± 1.31
200	−7.81	−1.11	4.71	2.25	22.15	5.07	± 2.92	± 1.40

Table 2-2 Correlation and results for the regression analysis for indirect and direct intra-abdominal pressure measurements by bladder infusion volume. **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.

Bladder instillation volume (mL)	Pearson's correlation (r)	P value	r^2 (%)	Standard error
0	0.39	0.45	15.0	4.5
50	0.38	0.31	14.7	3.58
100	0.58	0.08	33.6	4.09
200	0.51	0.14	25.5	4.31

Figure 2-1 Method for determination of the site for placement of the cannula for direct intra-abdominal pressure measurement. The height of the center of the tuber ishii (A) and the cranial eminence of the greater tubercle (B) were determined using a line perpendicular to the ground with the horse standing square. The site (X) of entry into the abdomen in the right flank was the point midway between A and B. **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.
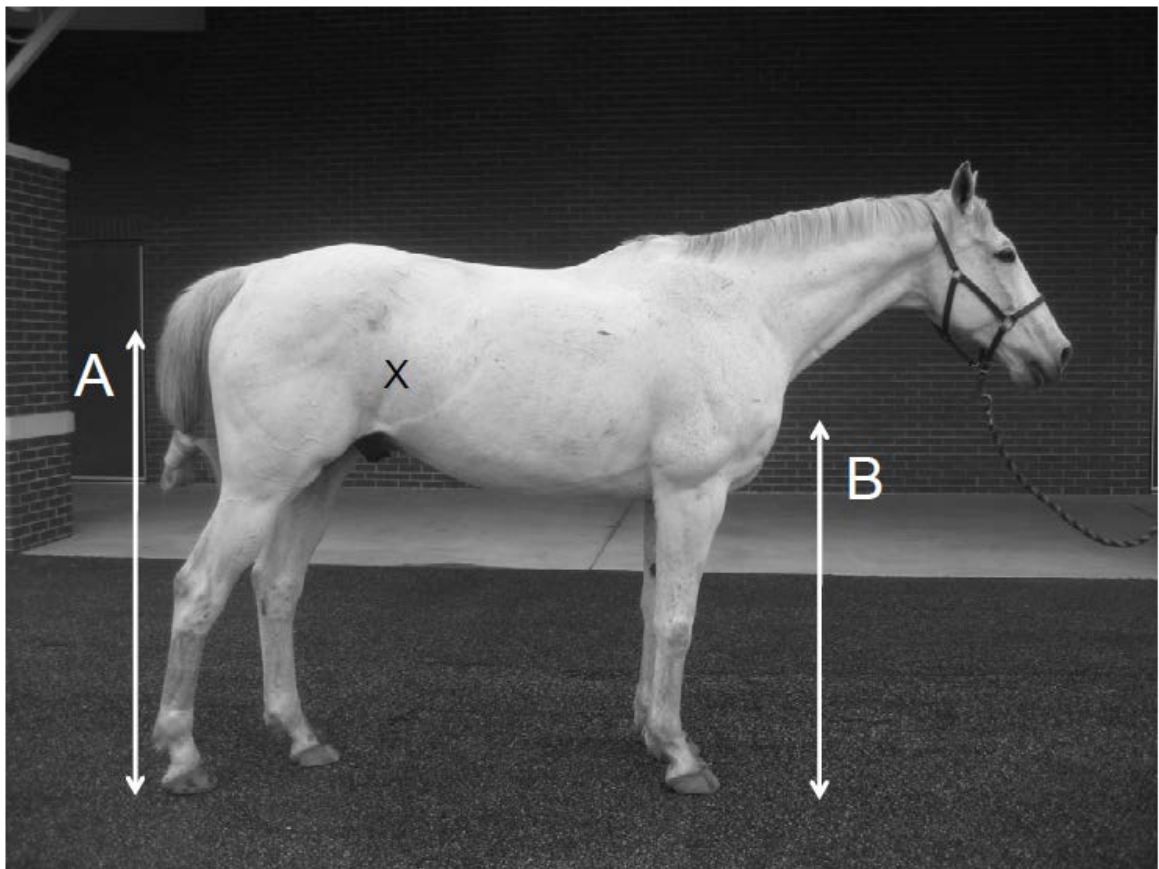
Figure 2-2 Horse instrumented for direct and indirect intra-abdominal pressure measurements. A Foley catheter is placed in the bladder, and clamped at the vulva to prevent air entry into the bladder (the manometer tubing for indirect pressure measurement is not attached in this picture). The arrow denotes the site of placement of the cannula for direct intra-abdominal pressure measurement. Inset shows detail: the 8-cm metal cannula (A) connected by an extension set (B) and 20 gauge needle (C) to the manometer tubing (D). The manometer was zeroed at the site of cannula entry into the flank for direct pressure measurement, and the tuber ishii for indirect pressure measurement. **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.
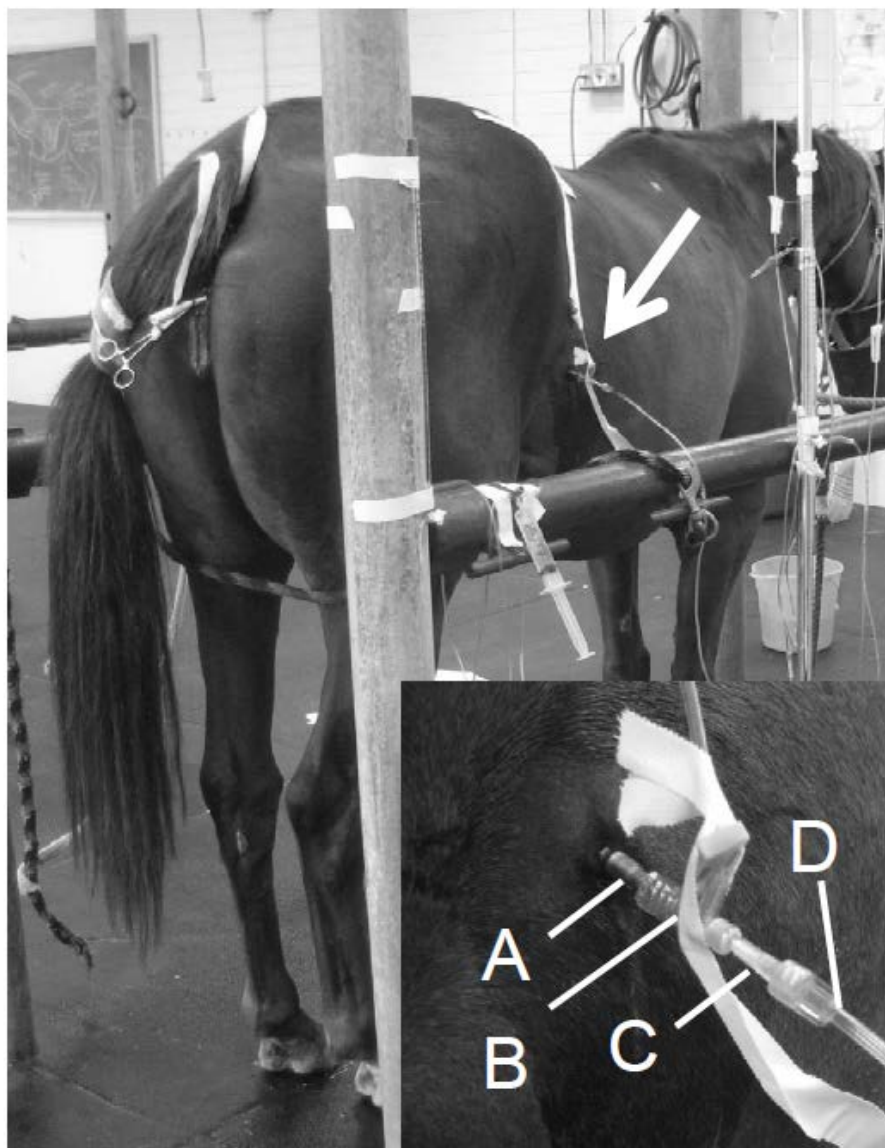
Figure 2-3 Modified Kron instrumentation for indirect intra-abdominal pressure measurement. A fluid reservoir (A) is connected to the water manometer (B) by an IV infusion set. The water manometer is connected to a 3-way stopcock and syringe (C) by pressure tubing. The syringe allows for a measured volume to be removed from the reservoir and infused into the bladder. The stopcock is attached to either a Foley or stallion urinary catheter by additional pressure tubing and a tubing adaptor (D). **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.
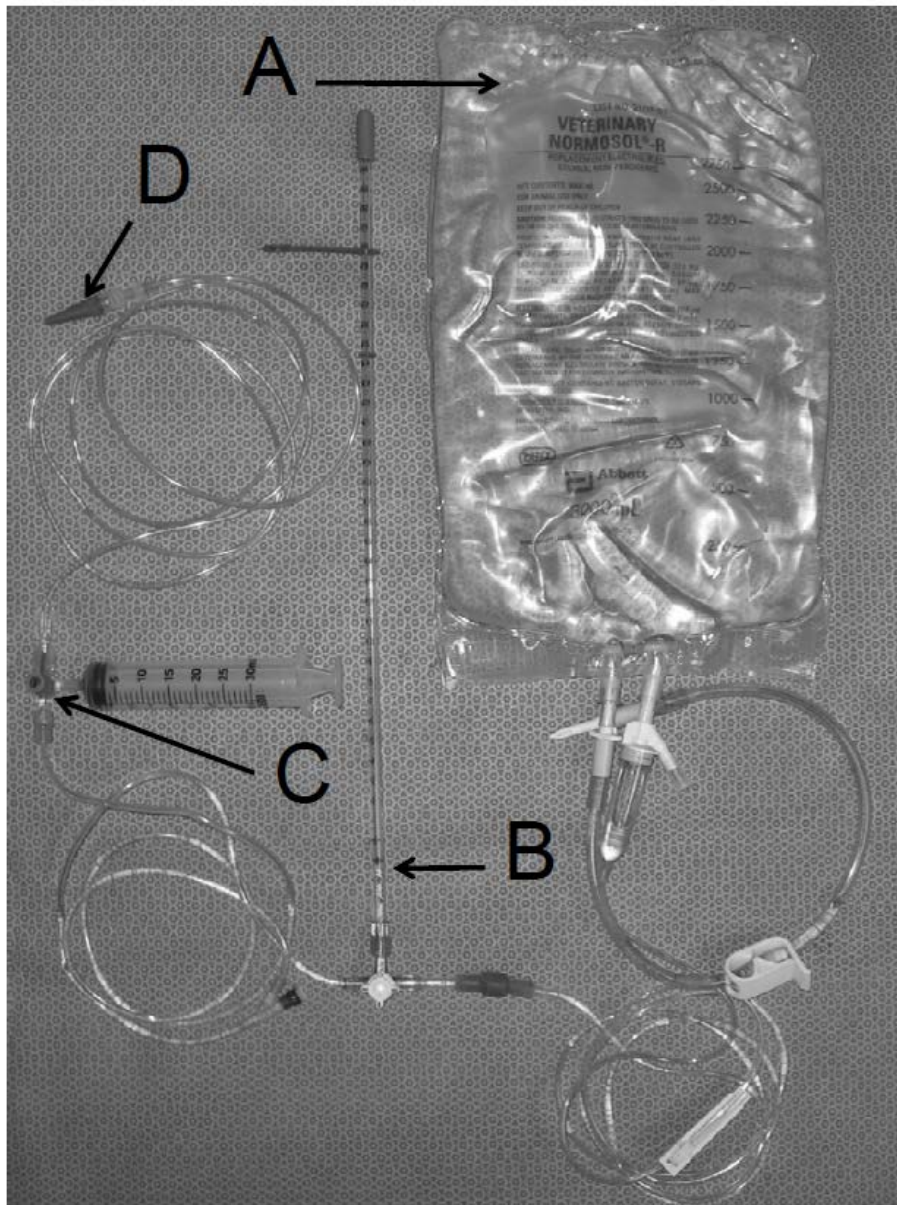
Figure 2-4 Correlation between body weight and the averaged direct intra-abdominal pressure for each horse. Direct pressures are measured using an intra-peritoneal cannula. A significant negative effect of body weight is noted (P=0.04). **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.
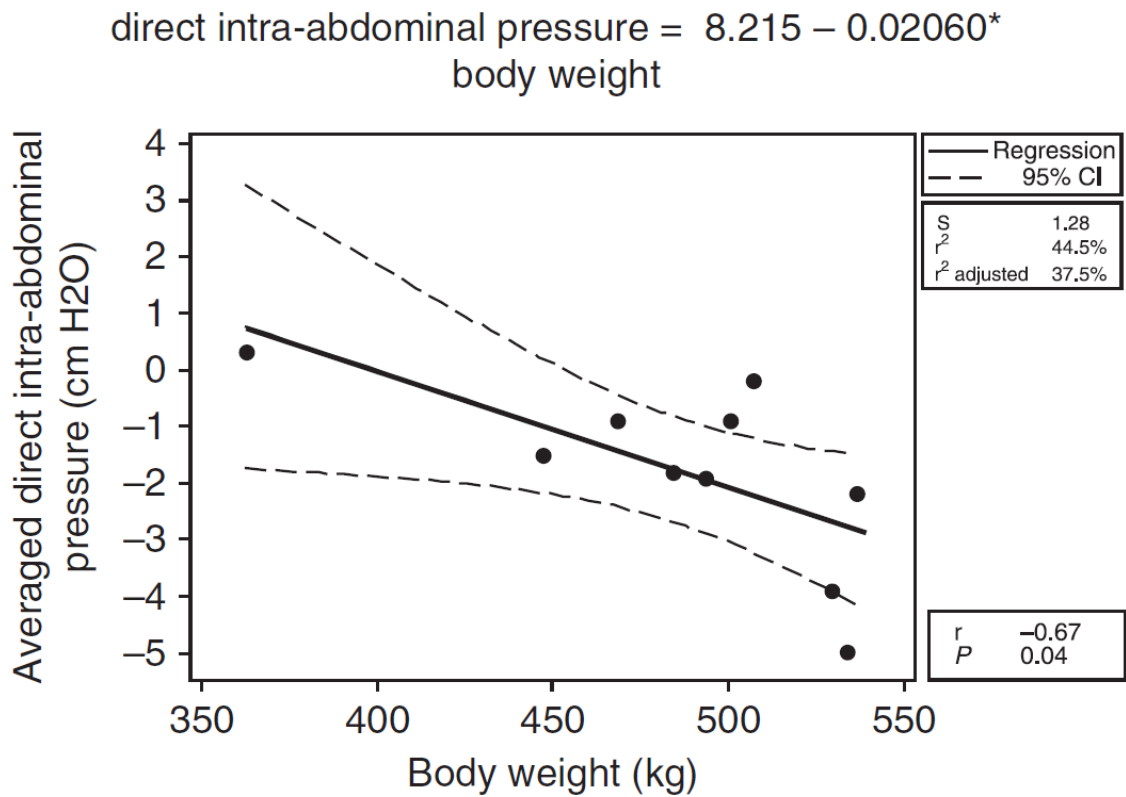
Figure 2-5 Comparison of the least squares means of the direct and indirect intra-abdominal pressure measurements as bladder infusion volume increases from 0 to 200 mL. Increasing bladder infusion volumes showed an effect on indirect pressures measured. Using Bonferroni's correction, a significant effect (denoted by *) of the infusion volume on indirect pressure was noted when 100 mL of fluid was infused in the bladder ($P = 0.004$) when compared with baseline. **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.
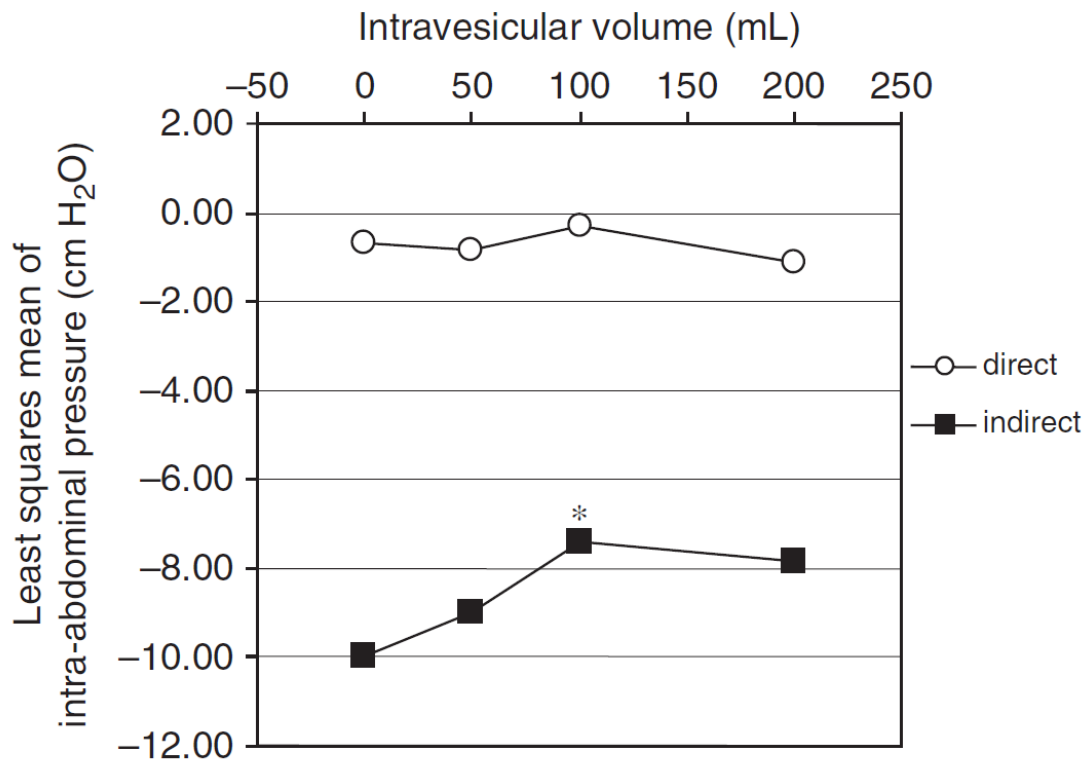
Figure 2-6 Correlations of the averaged direct and indirect intra-abdominal pressures for each horse for each volume of fluid infused in the bladder (panels A–D). A suspected outlier (direct intra-abdominal pressure of -5.47 cm H_2O and indirect pressure of -8.19 cm H_2O) was noted when 50 mL of fluid was infused into the bladder. A Cook's distance measure and calculation of difference of fit supported deletion of this value from the correlation. Low to moderate correlation was noted for the comparisons, but statistical significance ($P < 0.05$) was not met. **Published in Munsterman AS, Hanson RR.** Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care* 2009;19:545-553.

A

0 mL infusion volume
indirect intra-abdominal pressure =
−8.672 + 1.174 * direct intra-abdominal pressure

	Regression
—	95% CI
S	4.50
r²	15.0%
r² adjusted	0.0%

| r | 0.39 |
| P | 0.45 |

B

50 mL infusion volume
indirect intra-abdominal pressure =
−8.658 + 1.361 * direct intra-abdominal pressure

	Regression
—	95% CI
S	3.58
r²	14.7%
r² adjusted	2.5%

| r | 0.38 |
| P | 0.31 |

C

100 mL infusion volume
indirect intra-abdominal pressure =
−7.048 + 1.305 * direct intra-abdominal pressure

	Regression
—	95% CI
S	4.09
r²	33.6%
r² adjusted	25.4%

| r | 0.58 |
| P | 0.08 |

D

200 mL infusion volume
indirect intra-abdominal pressure =
−6.650 + 1.056 * direct intra-abdominal pressure

	Regression
—	95% CI
S	4.31
r²	25.5%
r² adjusted	16.2%

| r | 0.51 |
| P | 0.14 |

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Chapter 3

Abstract

Intra-abdominal pressure measurement in horses is currently validated using an intra-peritoneal cannula that directly measures intra-abdominal pressures from the abdominal cavity. A less invasive technique would be desired to reduce risks associated with an abdominal puncture. An indirect method of pressure measurement has been described in other species using a nasogastric tube to estimate pressures in the abdomen using pressures obtained from the gastric lumen. The purpose of this study was to develop and validate an indirect method of intra-abdominal pressure measurement using a nasogastric tube in normal standing horses. The hypothesis was that pressures within the stomach would correlate with pressures obtained simultaneously from the peritoneal cavity. The experiment was performed in ten, healthy, standing adult horses. Gastric pressures were obtained from a nasogastric tube using a U-tube technique. Pressures were obtained directly from the peritoneal cavity using a cannula and compared to the gastric pressures using increasing volumes of fluid infused into the stomach lumen. Comparisons were make using a Bland-Altman analysis and a Lin's concordance correlation coefficient. The average gastric pressure obtained in these horses was positive, while the

average direct peritoneal pressure was subatmospheric, with a mean bias of 15.9 +/- 5.3 cm H_2O. The variance of the gastric pressure measurements was significantly larger than that of the direct intra-abdominal pressure measurements, both within and between individuals. The Lin's concordance correlation coefficient demonstrated no correlation between the two techniques (p_c: -0.003). These results indicate that gastric pressure cannot be substituted for intra-peritoneal measurements of intra-abdominal pressure in normal, standing horses. Direct measurement of intra-peritoneal pressures is the most consistent method for measurement of intra-abdominal pressures due to the low variance of the technique, and should continue to be used for comparisons of intra-abdominal pressures within and between horses.

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3.1   Introduction

Intra-abdominal pressure (IAP) is the direct expression of the abdominal volume coupled with compliance of the abdominal body wall.[8] As the volume within the abdominal cavity increases, the compliance tends to decrease, eventually resulting in exponential increases in IAP for minor changes in intra-abdominal volume.[8,209] This loss of compliance along with abnormally high IAP is termed intra-abdominal hypertension (IAH).[8,197,199,210]

IAH may result directly from conditions affecting the abdomen, including surgery, trauma, gastrointestinal disease, peritonitis, ascites, and circumferential bandaging.[1,54,56] While transient elevations in abdominal pressure may be benign, persistent elevations may cause cardiovascular derangements, alter lymphatic drainage, and reduce tissue perfusion, contributing to multiple organ failure; this is termed abdominal compartment syndrome (ACS) in human medicine.[31,211-217] Although IAH has been recognized in horses, there is currently no consensus on a method of measurement of IAP in the horse.[10]

Two methods for IAP measurement have been described in horses.[10,11,148,159,201] The indirect method of measurement uses a urinary catheter placed in the bladder.[11,113,123,125,148] The bladder is used as a passive transducer, allowing the fluid within this organ to reflect the pressure within the abdominal cavity. The pressure of the urine within the bladder, plus a small instillation volume to prime the system, is used to estimate IAP. Although this method has been shown to correlate well in humans with pressures measured directly from the abdomen,[131,152] the pressures obtained from the bladder have been shown to vary widely when compared between horses,[11] precluding

the development of reference intervals for IAH in horses. Because of inter-horse variability, bladder pressures are currently recommended only as a screening tool in horses, and for measuring trends.

The second method of IAP measurement evaluated in the horse uses a needle, catheter, or cannula directly placed in the abdominal cavity.[10,11] The main disadvantage of the intra-peritoneal method is the degree of invasiveness; however, IAP obtained are less variable for repeated measurements within a horse, and more consistent between horses.[11] In horses, intra-peritoneal pressure measurement has been used in both clinical and research settings;[10,11] however, in clinical patients a less invasive method would be preferred.

In human medicine, an indwelling nasogastric tube has been used as a reliable method for indirect IAP measurement, when compared with bladder pressures.[113,160] The stomach acts in a manner similar to the bladder, allowing transduction of IAP to the fluid column established within the tube. Because horses are commonly intubated with a nasogastric tube, especially for diagnosis and therapy of gastrointestinal disorders, we sought to investigate this method as a non-invasive method of IAP measurement in the horse.

The objectives of this study were to describe a method for indirect IAP measurement using a nasogastric tube, and to obtain measurements using this technique in normal, standing horses. This method would be compared with an established method of IAP measurement using intra-peritoneal pressures to validate the results. The hypotheses were that IAP measurements could be obtained with a nasogastric tube, and

that this indirect method would be repeatable and comparable to previous methods of measurement for use in clinical equine patients.

3.2   Materials and Methods

3.2.1   Animal Selection

All procedures were approved by Auburn University's Institutional Animal Care and Use Committee (Protocol #2008-1587). Ten healthy adult horses, including 3 mares and 7 geldings, were used in this investigation. Mean weight was 529.1 +/- 40.98 kg. Breeds represented included 3 American Quarter Horses, 3 Thoroughbreds, 2 Tennessee Walking Horses, a Palomino, and a Hanoverian. The median age was 16 years (range 11–24 years). Each horse was fasted for 24 hours, with water withheld for the last 3 hours before the experiment. Fasting was instituted to reduce the effect of gastric fill on the measurements.

3.2.2   Instrumentation

On the day of the experiment, the horses were weighed, and restrained in the stocks. A physical examination was performed, and the horses were sedated with detomidine hydrochloride at a dose of 0.005-0.01 mg/kg, IV (Pfizer Animal Health, New York, NY). Direct intra-peritoneal pressure measurement was performed as described previously.[11] Briefly, the height of the midpoint of the tuber ishii and the height of the point of the shoulder were measured, and then averaged to obtain the height of a point midway between the two reference points. This measurement was used as the height for

insertion of the peritoneal cannula, and the site was selected approximately 10 cm caudal to the last rib. The area was clipped, aseptically prepared, and the skin and subcutaneous tissues anesthetized with 2% mepivicaine hydrochloride (100 mg per horse; Pfizer Animal Health, New York, NY). A stab incision was made into the skin and subcutaneous tissues with a #15 blade, to allow for blunt introduction of a 4-cm teat cannula into the peritoneal cavity. The cannula was capped with a closed extension set (Macrobore extension set no. 19328-48, Hospira, Inc., Lake Forest, IL) before introduction to reduce entrainment of air. Sterile, water based lubricant (Surgilube, E. Fougera and Co., Melville, NY) was also applied after introduction of the cannula around the site of entry to reduce the entrance of air.

For intra-peritoneal pressure measurement, a 20-gauge needle connected to a water manometer (CVP manometer, Mila International, Inc., Erlanger, KY) was introduced into the injection port of the extension set and into the cannula lumen. A solid fluid column was established from the manometer to the abdomen using a balanced electrolyte solution (0.9% sodium chloride injection, Hospira, Inc., Lake Forest, IL), and readings were obtained by positioning a 3-way stopcock open to the manometer. The manometer was zeroed at the height of insertion of the cannula.

For indirect IAP measurement using gastric pressures, a U-tube manometry technique was used based on a previously published method.[128] A standard equine nasogastric tube (12 mm internal diameter, 295 cm long) was placed in the stomach to the level of the 11th rib space, with the distance measured before placement of the tube. Air was removed from the stomach, and the tube was kinked and then capped to reduce the entrance of room air. For gastric pressure measurement, a solid fluid column was

established with the residual fluid in the stomach by infusion of a specific volume of water using a funnel. Gastric pressures were measured by holding the nasogastric tube vertical to act as a manometer, and the pressure determined using a using a centimeter ruler, zeroed at the point of the shoulder. (Figure 3-1)

3.2.3   Intra-Abdominal Pressure Measurement

After instrumentation, measurement of IAP began approximately 30 minutes after sedation was administered. For comparison of the intra-peritoneal and gastric pressures measurement techniques, pressures were recorded using both methods simultaneously after infusion of 5 different volumes of fluid (0, 400, 1,000, 2,000, and 3,000 mL) into the stomach. Multiple volumes were selected to determine the effect, if any, on gastric pressure, as well as to determine the optimum volume to consistently obtain a manometry reading. Each volume was tested in increasing order, due to the difficulty in removing fluid once it was instilled, so for each volume, the balance was added to what was infused previously in the stomach. Measurements were obtained after allowing the stomach to equilibrate for 5 minutes. A total of 6 repeated measurements were performed for each infusion volume. The measurements were obtained when IAP was lowest, and abdominal contractions were absent, noted in these horses to be at the end of inspiration, just before an observed active expiratory press.

3.2.4   Statistical Analysis

The average intra-peritoneal and gastric pressures were calculated for each horse, and for each volume of fluid infused into the stomach. The intra- and inter-individual variation was also determined for each method of pressure measurement. Measurements

of gastric pressure were evaluated using a Kruskal-Wallis test to assess the equality of the population medians for the infusion volumes tested. Agreement between the 2 methods of measurement for each infusion volume was determined using the method reported by Bland-Altman.[218] The bias was calculated for each stomach infusion volume for each horse by subtracting the pressures obtained from the peritoneal cavity from the gastric pressures. Relative bias was also calculated for each infusion volume as follows: (gastric pressure - intra-peritoneal pressure)/ (0.5 x [gastric pressure + intra-peritoneal pressure]) x 100. A positive absolute bias or relative bias reflects an overestimation of the intra-peritoneal pressures by the gastric method as compared with the intra-peritoneal method and vice versa. Limits of agreement were reported as bias or relative bias +/- (1.96 x standard deviation). Normality and equality of variance were assessed using the Kolmogoroz-Smirnov and the Levine tests, respectively. Concordance between the 2 methods of measurement was assessed using Lin's concordance correlation coefficient (p_c), to evaluate 2 techniques measuring the same variable without establishing a gold standard.[219,220] A p_c of 1 or -1 indicates perfect concordance, whereas a p_c of 0 indicates no correlation. All statistical analyses were performed using commercially available statistical software packages (SAS Analytics Pro, SAS Institute, Inc., Cary, NC; Minitab 15 Statistical Software, Minitab, Inc., State College, PA). Statistical significance was set at P< 0.05.

3.3   Results

A total of 300 pairs of IAP measurements were attempted in the 10 horses, with 6 paired measurements taken at each of the 5 infusion volumes. However, only 264 paired

readings were obtained due to loss of the fluid column in the nasogastric tube preventing the measurement of gastric pressure. IAP obtained by the peritoneal cannula for all infusion volumes ranged from -6.6 to 3.1 cm H_2O (mean +/- standard deviation, -1.59 +/- 2.09 cm H_2O), and the average pressure calculated at each volume infused was subatmospheric. (Table 3-1) Serial measurements of intra-peritoneal pressures produced consistent readings within and between horses (intra-individual variance range: 0.05 to 0.10 cm H_2O; inter-individual variance range: 2.88 to 5.74 cm H_2O).

Gastric pressures, in comparison, ranged from 0 to 25.8 cm H_2O (mean +/- standard deviation: 14.44 +/- 4.69 cm H_2O). (Table 3-2) Measurements of gastric pressures appeared repeatable within each horse (variance: 0.34-0.91 cm H_2O); however, the inter-individual variation for gastric pressures was 4 to 10 times the magnitude of the variation between horses for intra-peritoneal pressures. At all instillation volumes, both intra- and inter-individual variation was significantly greater for gastric pressures when compared with intra-peritoneal pressures (F critical value: 3.2, 95% confidence level). Comparison of gastric pressures obtained using each of the 5 different stomach infusion volumes noted no significant effect of infusion volume on the pressures obtained (P=0.68).

The mean bias mean +/- standard deviation between the 2 methods of pressure measurement was 15.9 +/- 5.3 cm H_2O (95% CI: 5.47 to 26.33 cm H_2O). (Figure 3-2) The mean relative bias (+/- standard deviation) was 244.3 +/- 199.2% (95% CI: 184.5 to 304.2%). The assumption of normality was questionable (P=0.02); however, the equality of variance was constant (P=0.89) indicating the magnitude of bias was not dependent on gastric volume. The concordance correlation coefficient (p_c) between intra-peritoneal and

gastric pressures for all infusion volumes was -0.003 (95% CI: -0.02 to 0.02). Among

individual infusion volumes, a volume of 1,000 mL produced the largest p_c (0.009), but

this value was not significantly different from the concordance correlation coefficients at

the other volumes. None of the P-values for the correlation coefficients reached statistical

significance (overall P=0.75).

3.4   Discussion

In this study, we assessed the viability of the use of a nasogastric tube to obtain

indirect IAP in the normal, standing horse through evaluation of bias and precision

compared with a known method of direct abdominal pressure measurement using intra-

peritoneal pressures. Although we were able to obtain gastric pressure measurements in

all of the horses, the intra- and inter-individual variation was significantly greater for

gastric pressure measurements at all infusion volumes when compared with intra-

peritoneal pressures (Tables 3-1 and 3-2). Comparison between the 2 methods of pressure

measurement demonstrated a conclusive lack of correlation (Lin's concordance

correlation coefficient: -0.003; P=0.75), indicating that gastric pressures should not be

used in place of intra-peritoneal pressures for IAP measurement.

A U-tube manometry technique was used to measure gastric pressures in horses in

this study. This method uses the tubing itself as the manometer and has been validated in

pigs and in humans as a non-invasive method for IAP measurement.[131,160] By raising the

tubing vertically, a U is formed, allowing the water pressure to be measured as the height

of the column of fluid above a given reference. In our horses, the point of the shoulder

was chosen as a repeatable reference point to approximate the level of the stomach in the

horse. This manometry method was selected for its simplicity, as well as in an effort to reduce the amount of specialized equipment needed for IAP measurement.

Although this method was able to obtain relatively repeatable pressure measurements within each horse, there was a high amount of inter-individual variability, and a complete lack of correlation with pressures measured from the peritoneal space. In people, gastric pressures have been suggested to be directly influenced by the migrating motor complexes of the stomach musculature,[221] as well as ileus or enteral feeding.[165] In horses, the stomach has been shown to contract up to 2–3 times a minute during stage 3 of the migrating motor complex, indicating similar contractions may have resulted in the variations in gastric pressure measurements noted in our study.[222] While ileus was absent and enteral feedings were not being administered in these horses, it is reasonable to assume that these conditions may also increase the volume in the stomach and the pressures recorded, and would have to be considered as an additional variable in clinical cases.

The gastric instillation volumes tested in this experiment were also a potential source of variability in the measurement of gastric pressures. It is known that bladder infusion volumes can increase measured pressures due to activation of the detrusor muscle by distention of the organ.[136,137,139,152,202,203] It was possible that a similar effect could occur due to activation of a gastric motor response. While increasing gastric instillation volumes in the current study resulted in a trend toward increasing stomach pressure measurements (Table 3-2), there was no significant effect of infusion volume on pressure (P=0.68). Based on these data, we can only recommend that installation volumes for measurement of gastric pressures be > 0 mL, to allow for a consistent fluid column

for a manometry reading. Further studies would be required to investigate the effects of larger volumes on gastric pressures.

The most likely cause of the inaccuracies of the pressures recorded was the fluid manometry method itself.[113] The residual volume in the stomach was unknown, which raises questions as to the accuracy and reproducibility between measurements based on a specific volume infused. The residual volume may have been reduced due to normal gastric emptying, or may have been increased by reduced motility caused by administration of an alpha-2 agonist or the presence of an indwelling nasogastric tube.[223,224] In an effort to minimize these effects, gastric pressures were obtained within 5 minutes of fluid instillation and all measurements were completed within 30 minutes to 1 hour of the initial measurement. The likelihood that gastric emptying had an effect on residual volumes or gastric pressures remains unanswered and will require further investigation.

In addition, it is impossible to know exactly where the tubing is placed in the horse's stomach, and any air pocket in the stomach or tubing can attenuate the pressures transferred through the fluid column.[113] The U-tube method, as described, requires complete drainage of the stomach and removal of any residual air in the organ before measurement to avoid this complication,[128,160] but this was difficult to perform or confirm in the horses used in this experiment, and the authors know of no reasonable methods that could be used to do so. This may be the reason why the water instilled was occasionally able to drain completely into the stomach, causing a loss of the fluid column, and the inability to obtain gastric pressures. Out of 300 attempts, the fluid column was lost 36 times, giving a success rate of 88% for gastric pressure measurement using this method.

In order to avoid the pitfalls of the manometry system, the use of a gastric tonometry balloon in human medicine has been shown to increase accuracy, and allow for the measurement of gastric pH as an independent measure of perfusion.[163,165] The balloon is filled with a specific volume of air, and pressure changes are recorded by measuring compression of the balloon. This air-filled system negates the problems with the manometry technique in terms of the variability of an arbitrary reference point, or over- and under-damping of the system by air bubbles. Although gastric tonometry has been described in foals,[225,226] a specialized catheter would have to be designed to reach an adult horse's stomach, because commercially available human catheters measure only 46 inches.[227]

In adult horses, the use of a barostatic bag, which measures changes in pressure by recording the amount of air that must be injected or removed to maintain a pre-set pressure, has been used in place of the tonometry balloon for gastric pressure measurement.[228] This method has been used to monitor changes in stomach pressures in exercising horses; however, direct comparison to intra-peritoneal pressures or any other method of indirect pressure measurement has not been performed. Both of these substitute methods for gastric pressure measurement (barostatic bags and gastric tonometry) would still be affected by the migrating motor complex,[113] but may warrant investigation as less invasive options for intra-abdominal pressure measurement using gastric pressures.

Direct IAP measurements obtained in this study from the peritoneal cavity compared favorably with our previous study, which measured intra-peritoneal pressures as well as indirect pressures measured using a bladder catheter.[11] Intra- and inter-

individual variance for intra-peritoneal pressure was similar in both reports, indicating that the use of this method appears to provide reliable results in normal horses. Both studies noted a lack of correlation between the indirect measurements using bladder or gastric pressures and intra-peritoneal pressures. In addition, there was significantly greater inter-individual variation for the indirect measurement of IAP regardless of technique. What we can conclude is that both methods of indirect IAP measurement are highly variable between horses, which will make statistical comparison between horses and between studies difficult. At the present time, standard reference intervals for diagnosis of IAH may only be possible based on direct intra-peritoneal pressure measurement.

Risks using a nasogastric tube for gastric pressure measurement include pharyngitis, ethmoid hemorrhage, and esophageal perforation if the tube is indwelling. No complications were noted in the horses used in this study, and the horses tolerated the procedures with little indication of discomfort, indicating the nasogastric intubation procedure would be a viable one, if a better instrument for measurement is developed. The use of a non-invasive, indirect method would be preferable to the more invasive intra-peritoneal procedure, to avoid possible complications including enterocentesis, peritonitis, and local subcutaneous inflammation or abscess formation.

In this study, we have demonstrated that intra-gastric pressure measurements using a U-tube manometer in this group of normal, standing horses do not correlate with pressures measured directly from the peritoneal cavity. Although the measurements were relatively repeatable in each horse, the variation was significantly greater than the direct intra-peritoneal method when comparing pressures between horses, indicating that direct

measurement of IAP may be more accurate when establishing reference intervals for IAH in the horse. Investigations into novel methods using piezoelectric probes or gastric tonometry are warranted to reduce the inherent risks of the more invasive method.

Table 3-1 Comparison of intra-peritoneal pressures for each gastric infusion volume. **Published in Munsterman AS, Hanson RR.** Evaluation of gastric pressures as an indirect method for measurement of intraabdominal pressures in the horse. *J Vet Emerg Crit Care* 2011;21:29-35.

Gastric infusion volume (mL)	N	Minimum (cm H$_2$O)	Maximum (cm H$_2$O)	Mean (cm H$_2$O)	SD (cm H$_2$O)	Variance within horse (cm H$_2$O)	Variance between horses (cm H$_2$O)
0	48	—5.2	0.3	—1.95	1.62	0.07	2.89
400	55	—6.1	1.0	—1.89	2.07	0.05	4.34
1,000	54	—6.6	1.0	—2.26	2.12	0.10	4.83
2,000	54	—6.4	1.7	—1.39	2.29	0.05	5.74
3,000	53	—2.7	3.1	—0.49	1.83	0.05	3.60
Overall	264	—6.6	3.1	—1.59	2.09	0.39	4.66

N, number of intraperitoneal pressure measurements.

Table 3-2 Comparison of gastric pressures for each gastric instillation volume. **Published in Munsterman AS, Hanson RR.** Evaluation of gastric pressures as an indirect method for measurement of intraabdominal pressures in the horse. *J Vet Emerg Crit Care* 2011;21:29-35.

Gastric instillation volume (mL)	N	Minimum (cm H$_2$O)	Maximum (cm H$_2$O)	Mean (cm H$_2$O)	SD (cm H$_2$O)	Variance within horse (cm H$_2$O)	Variance between horses (cm H$_2$O)
0	48	0.0	20.3	11.95	5.19	0.91	29.12
400	55	4.2	21.4	14.31	4.24	0.35	24.35
1,000	54	10.8	25.8	15.60	4.28	0.34	19.81
2,000	54	7.2	24.8	14.89	4.88	0.44	25.79
3,000	53	9.0	21.7	15.17	4.19	0.35	18.66
Overall	264	0.0	25.8	14.44	4.69	5.71	16.81

N, number of gastric pressure measurements.

Figure 3-1 Measurement of gastric pressures using a nasogastric tube as a U-tube manometer. The instillation volume has been administered and the tube has been taped vertically to the stocks to allow the pressure to be read as the height of the meniscus on the ruler, zeroed at the point of the shoulder. The arrow depicts the height of the fluid column in this picture. **Published in Munsterman AS, Hanson RR.** Evaluation of gastric pressures as an indirect method for measurement of intraabdominal pressures in the horse. *J Vet Emerg Crit Care* 2011;21:29-35.

Figure 3-2 Bland-Altman analysis of the intra-abdominal pressure measurements obtained concurrently use of an intra-peritoneal cannula and a nasogastric stomach tube. Values were measured repeatedly in each horse, and averaged for comparison. Mean bias is represented by the solid horizontal line, and the upper and lower limits of agreement (mean +/- [1.96 x standard deviation]) are represented by dashed horizontal lines. **Published in Munsterman AS, Hanson RR.** Evaluation of gastric pressures as an indirect method for measurement of intraabdominal pressures in the horse. *J Vet Emerg Crit Care* 2011;21:29-35.
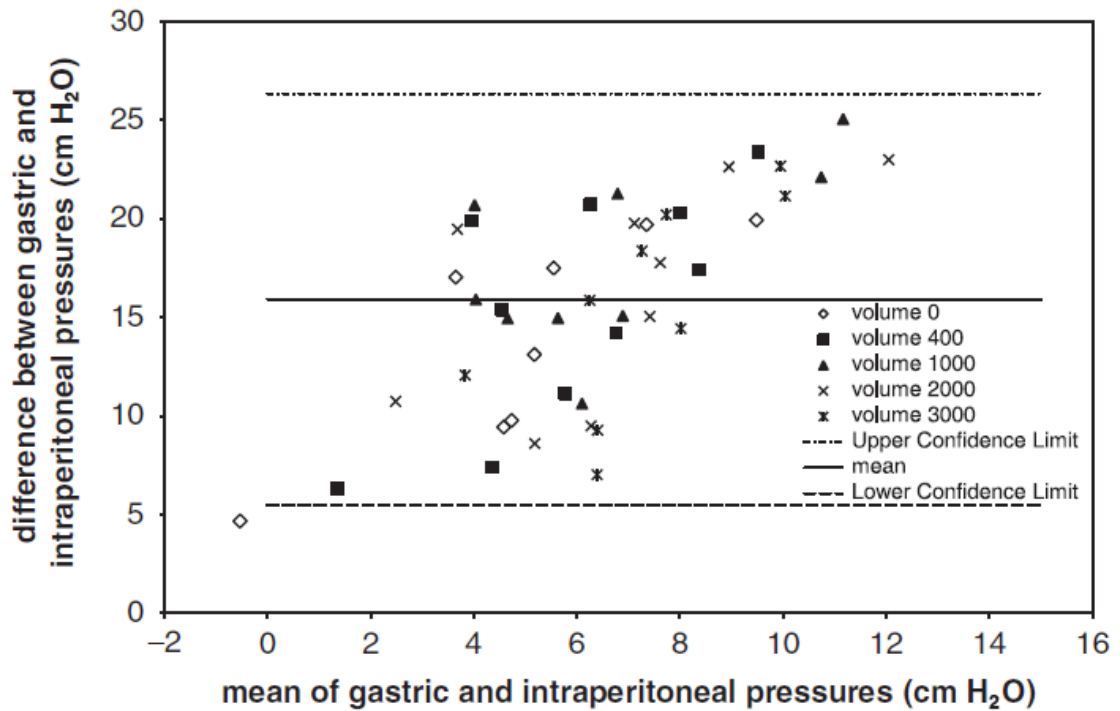
Chapter 4

Abstract

Current techniques to measure intra-abdominal pressures in horses use metal cannulas. There are concerns that the metal cannula could puncture abdominal viscera if left in place, and these concerns prevent continuous pressure measurements. The aim of this study was to validate the use of a solid piezo electric microsensor catheter and digital monitoring system for the measurement of direct intra-abdominal pressure in horses. This novel microsensor would be assessed by comparing its values with the ones simultaneously obtained by means of an intra-peritoneal cannula. Ten, healthy, adult horses had intra-abdominal pressures measured simultaneously through an intra-peritoneal cannula zeroed midway between the height of the tuber ishii and point of the shoulder and with an intra-peritoneal solid microsensor placed within the abdomen at the same level as the metal cannula. Three repeated intra-abdominal pressure measurements were obtained at rest, after placement of a nasogastric tube, and after placement of 5-L increments of water into the stomach, up to a total volume of 20 L of water. The mean difference between values obtained was 1.25 +/- 2.53 mmHg. The correlation coefficient was 0.825. Direct intra-abdominal pressure monitoring with a solid microsensor allows

continuous monitoring without concern for gastrointestinal perforation, is simple to use and to calibrate, and is minimally invasive.

4.1   Introduction

Intra-abdominal pressure (IAP) is related to compliance of the abdominal wall and the volume of abdominal contents. Excessive and/or sustained increases in pressures within the abdomen are termed intra-abdominal hypertension (IAH), which is a recognized pathologic condition in both humans and animals.[10,199] IAP above 40 cm H_2O may result in organ failure in affected human patients, and this condition is known as abdominal compartment syndrome (ACS).[199,229-232]

Current techniques for measuring abdominal pressures in human medicine consist of either direct (within the abdomen) or indirect (within the bladder or stomach) approaches using both rigid and malleable instrumentation. Typically, human patients monitored for IAH are on mechanical ventilation in a vegetative state or are under heavy sedation and unable to resist diagnostic procedures. Techniques for measuring IAP in humans may not be applicable for continuous monitoring of IAP in an awake, mobile equine patient.

The currently accepted method for measuring IAP in horses is a metal cannula that allows for measurements to be taken only while the horse is restrained and standing quietly over concerns that the metal cannula could dislodge or cause damage to abdominal viscera.[11] Additionally, the metal cannula can cause horses to react to the sensation and splint against it, which may increase measured pressures, as noted in human patients.[230] A solid piezo electric microsensor is attached to a malleable cable and has already been validated for use in humans for the measurement of intra-abdominal and intracranial pressures.[232] The purpose of this study was to validate the use of a solid

microsensor (Codman Microsensor ICP Transducer, DePuy Synthes, Raynham, MA) to allow for repeated, less invasive measurements of direct intra-peritoneal IAP in horses.

4.2   Materials and Methods

4.2.1   Animal Selection

All procedures were approved by Auburn University's Institutional Animal Care and Use Committee (Protocol #2011-1926). Ten healthy adult horses, including 3 mares and 7 geldings, weighing an average of 546 kg (range, 481-670 kg) with a median age of 17 years (range, 11-22 years) were used in this investigation. Breeds represented included Thoroughbred (3), Tennessee Walking Horse (1), Arabian (2), Warmblood (2), American Quarter Horse (1), and American Paint Horse (1). Each horse was fasted for 24 hours before the initiation of the procedure, with water being withheld for the last 3 hours. Fasting was instituted to reduce the effects of intestinal fill on the pressure measurements, and water was withheld to minimize alterations in gastric volume.

4.2.2   Instrumentation

On the day of the procedures, the horses were weighed and restrained in stocks, and vital parameters including heart rate, respiratory rate, temperature, gastrointestinal borborygmi, digital pulses, mucous membrane color, and capillary refill time were obtained. The horses were then sedated with detomidine hydrochloride (0.01 mg/kg, IV; Pfizer Animal Health, Exton, PA). Direct IAP measurements were acquired using two methods. The first method was a modified abdominocentesis procedure previously

described.[11] Briefly, the height of the midpoint of the tuber ishii and the height of the point of the shoulder were measured and then averaged to obtain the height of a point midway between the two reference points. This measurement was used as the height for insertion of the teat cannula into the abdomen, approximately 8 cm caudal to the last rib on the right side of the horse. The selected area was clipped and aseptically prepared, and the skin and subcutaneous tissues were anesthetized with 2% mepivacaine hydrochloride (100 mg per horse; Pfizer Animal Health, Exton, PA). A stab incision was made into the skin and subcutaneous tissues with a number 15 blade to allow for blunt introduction of a 10-cm teat cannula into the peritoneal cavity. Entrance into the peritoneal cavity was confirmed by the loss of resistance to pressure, as felt by the operator when the cannula passed through the peritoneum. The cannula was flushed and capped with a closed extension set before introduction to reduce entrainment of air. Sterile, water-based lubricant was also applied after introduction of the cannula around the site of entry to reduce the entrance of air.

For IAP measurement using this cannula, the extension set was connected to a water manometer. A solid fluid column was established from the manometer to the abdomen, using balanced electrolyte solution, and readings were obtained by positioning a three-way stopcock open to the manometer. The manometer was zeroed at the height of insertion of the cannula, and the horse's head was maintained at the level of the withers throughout the experiment.

The second method used a solid microsensor (Figure 4-1A) placed at the same height as the cannula at a location 14 cm caudal to the last rib, again on the right side of the horse. Prior to placement within the abdomen, the microsensor was zeroed within a

fluid medium (0.9% sodium chloride solution, Baxter Healthcare Corp., Deerfield, IL), as directed by the manufacturer. An open-ended metal cecal cannula (Figure 4-2) was placed intra-peritoneally at this location in the same fashion as the first metal cannula. The obturator was removed, and the microsensor was fed through the cannula into the abdomen. After the cannula was fed 12 cm into the abdomen, it was removed from the body wall and secured to the stocks, leaving only the microsensor in place within the abdomen. The microsensor was then connected to an intra-cranial pressure monitoring device (Codman Microsensor Transducer Monitor, DePuy Synthes, Raynham, MA) that displayed the measured pressures in mm Hg. (Figure 4-1B)

4.2.3 Intra-Abdominal Pressure Measurement

After instrumentation, simultaneous measurement of IAP from both of these methods began approximately 30 minutes after sedation was administered. Values for IAP were recorded at end-inspiration when abdominal muscles were relaxed, and three separate measurements were taken at each observation. IAP were first measured at rest. A premeasured nasogastric tube was then passed through the left nostril to the level of the stomach. Five minutes were allowed to elapse after passage of the nasogastric tube to allow abdominal pressure to equilibrate, and then measurements were taken before infusing water into the stomach. Next, 5 L of water was instilled into the stomach using a hand pump, and the end of the nasogastric tube was capped. Again, 5 minutes were allowed to elapse prior to IAP measurements being taken. The same procedure was repeated after a total of 10, 15, and 20 L of water had been instilled into the stomach. All measurements from instillation of the initial 5 L of fluid to the final measurement with a

total of 20 L instilled into the stomach were obtained in less than 30 minutes. After the final pressure measurements were obtained, excess water was siphoned off the stomach and the nasogastric tube removed. This method of gastric distension has previously been shown to cause significant increases in IAP.[233]

4.2.4   Statistical Analysis

A total of 180 IAP measurements were taken for this study to evaluate the correlation between the two methods of abdominal pressure measurement. Abdominal pressures were averaged for each of the triplicate measurements and the numbers analyzed by a commercial statistical program (Minitab 16 Statistical Software, Minitab, Inc., State College, PA). Data were found to be normally distributed by using an Anderson Darling test. A Pearson's correlation coefficient was calculated to determine the linear fit between the two data sets. A Bland-Altman plot was constructed to evaluate the limits of agreement of the two methods.

4.3   Results

Mean IAP +/- standard deviation for each of the methods of abdominal pressure measurement at each level of fluid instillation is shown in Table 4-1. The correlation coefficient between the mean pressures obtained by the microsensor and those measured by the manometer was 0.825. This value shows the microsensor correlates well with the currently accepted method of measuring intra-abdominal pressure in the horse. A Bland-Altman plot of the data found no bias between the two data sets. (Figure 4-3) The limits of agreement (LOA) for the data were 6.32 mmHg and -3.81 mmHg, values that are

clinically acceptable. The Bland Altman plot found the mean difference and standard deviation of the differences between the two data sets was 1.25 +/- 2.53 mmHg.

4.4   Discussion

IAP measurement in horses will not become common-place in referral institutions until a method has been developed where we can safely monitor trends in IAP over time. Human physicians use IAP measurements to determine when to proceed with surgical intervention for IAH. The goal would be to establish reference values and a safe and effective procedure to allow for similar applications in equine medicine. The previously described method involves a metal teat cannula, leading to concerns over abdominal viscera or abdominal wall damage. The metal teat cannula also allows only intermittent measurements of abdominal pressure, which encouraged the evaluation of newer technology. A solid microsensor, currently being used in human medicine, was selected to determine if the same technology could be used in horses. This microsensor allows for continual monitoring and recording of pressures from the instrumented patients.

Our study found the microsensor method correlated well with the currently accepted technique. Some variation in pressure measurements may have been introduced by differences in the height in the abdomen of the tip of the microsensor catheter compared to the cannula. In addition, the water manometer technique has been noted to have inherent variability in both the water system itself and human error in reading the pressures. Both of these innate issues will likely prevent perfect correlation between the two.

Advantages of the microsensor are that the malleable cord will allow us to take continuous direct IAP measurements of horses without continuously re-cannulating the abdomen and without the need for restraint in stocks. Additionally, the unit is simple to use and allows digital recording of any measured data. It is possible that leaving an instrument within the abdominal cavity of a horse for a prolonged period may pose a risk for the development of peritoneal sepsis or implant infection. Neither of these complications has been reported in humans, where the device in commonly used to measure both intra-cranial and intra-abdominal pressures.[232]

In conclusion, the strain gauge microsensor is a valid method for measuring direct intra-peritoneal IAP in horses and circumvents some of the possible complications associated with the metal teat cannula previously used for measuring IAP.

Table 4-1 Mean intra-abdominal pressure +/- standard deviation measured at each volume of gastric fluid instillation for the individual methods of measurement. **Published in Barrett, E.J., Munsterman, A.S., Albanese, V. et. al.** Direct Measurement of Intra-Abdominal Pressures in a Horse by Using a Solid Microsensor. *Journal of Equine Veterinary Science* 2013;33:1000-1003.

Gastric Instillation Volume	Metal Cannula Measurements (mm Hg)	Microsensor Measurements (mm Hg)
Baseline	-4.09 ± 1.66	-3.73 ± 2.30
Postnasogastric intubation	-3.41 ± 1.74	-2.76 ± 2.49
5 L	-2.30 ± 1.68	-0.93 ± 1.79
10 L	-1.34 ± 1.70	0.73 ± 1.88
15 L	-0.09 ± 1.78	1.90 ± 2.53
20 L	1.66 ± 2.62	2.73 ± 2.18

Figure 4-1 Solid microsensor catheter (A) and digital monitoring system (B). **Published in Barrett, E.J., Munsterman, A.S., Albanese, V. et. al.** Direct Measurement of Intra-Abdominal Pressures in a Horse by Using a Solid Microsensor. *Journal of Equine Veterinary Science* 2013;33:1000-1003.
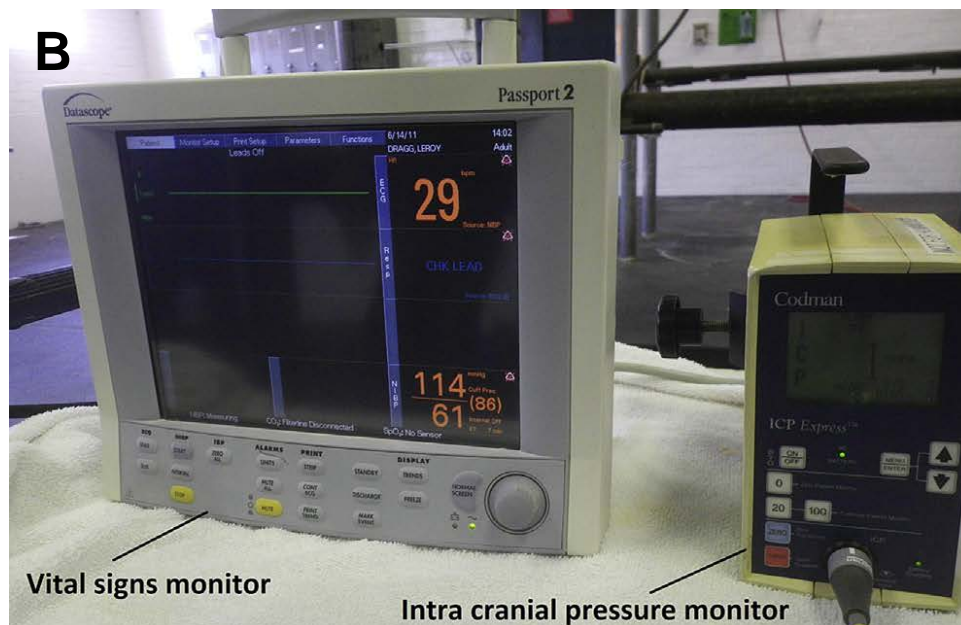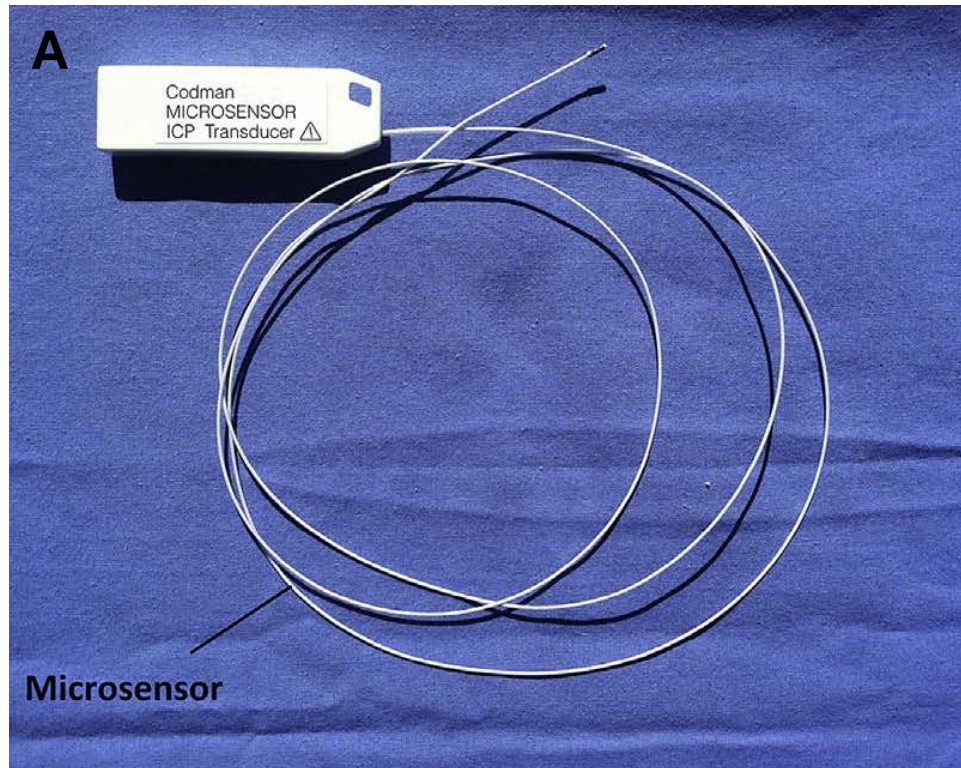
Figure 4-2 Open-ended metal cecal cannula. **Published in Barrett, E.J., Munsterman, A.S., Albanese, V. et. al.** Direct Measurement of Intra-Abdominal Pressures in a Horse by Using a Solid Microsensor. *Journal of Equine Veterinary Science* 2013;33:1000-1003.

Figure 4-3 Bland-Altman analysis of the intra-abdominal pressure measurements obtained concurrently through either the Codman catheter or the water manometer and metal cannula. Solid horizontal lines represent the upper and lower limits of agreement (mean +/- 2 x standard deviation). **Published in Barrett, E.J., Munsterman, A.S., Albanese, V. et. al.** Direct Measurement of Intra-Abdominal Pressures in a Horse by Using a Solid Microsensor. *Journal of Equine Veterinary Science* 2013;33:1000-1003.

Intra-abdominal pressures - microsensor vs metal cannula
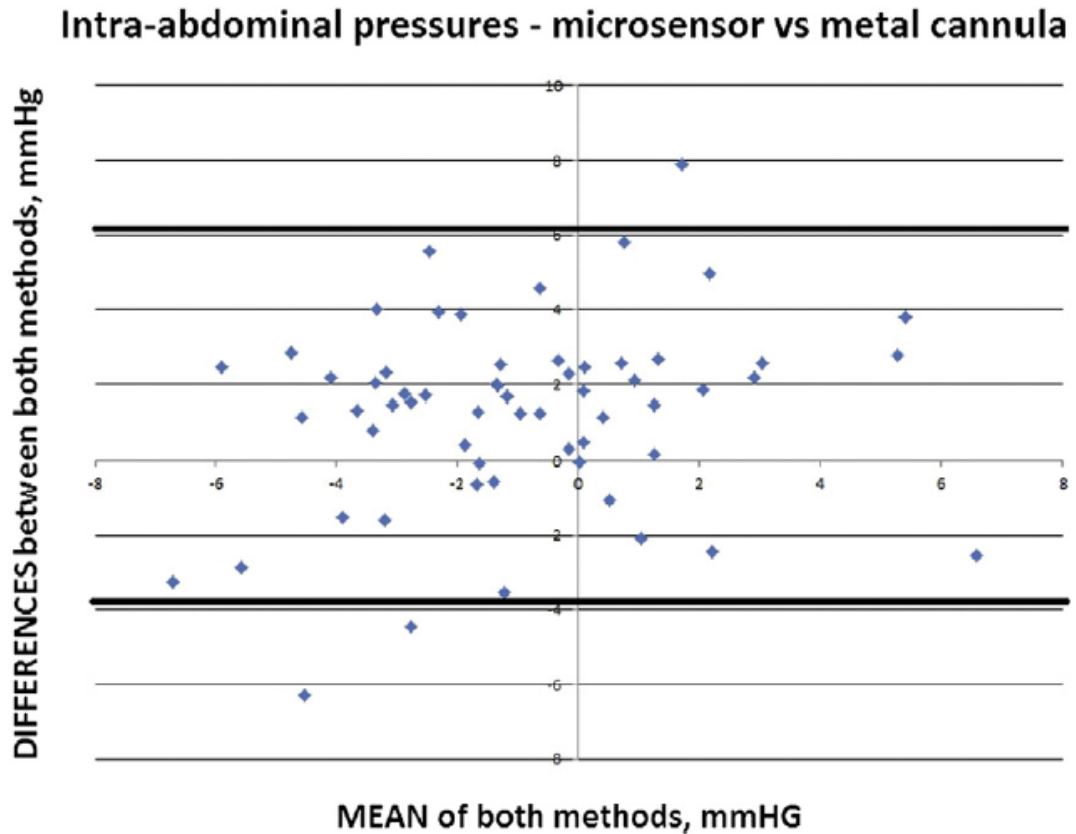
Chapter 5

5.1   Introduction

Central venous pressure (CVP) is the pressure measured from either the superior

or inferior vena cava close to the insertion on the right atrium, as a surrogate of the

pressure measured from the right atrium itself.[234] In the literature, right atrial pressure is

often referred to as CVP, although not technically correct. CVP is recommended as a

resuscitation endpoint in clinical cases of septic shock as a measure of preload and fluid

responsiveness.[64,235,236] However, the use of these resuscitation guidelines without

consideration of complicating external factors that may alter CVP has led to the

observations of decreased survival in patients resuscitated to the guidelines of the

Surviving Sepsis Campaign, compared to those who were under resuscitated.[67]

This is especially true in patients with IAH, as approximately 50% of the pressure

within the abdomen is transmitted across the diaphragm to the thoracic cavity.[85] CVP

measured in the presence of IAH will partially reflect the surrounding intra-thoracic

pressure, demonstrating a positive correlation with IAP, and negative correlation with

cardiac output.[74,237,238] The relationship between CVP and IAP is further complicated by

the hemodynamics of the abdominal cavity.[80,239] Early in the progression of IAH, venous

return increases as the abdominal organs are compressed and blood is forced from the

splanchnic circulation to the inferior vena cava. In this stage, CVP should mirror increases in IAP. Once IAP has reached a critical point, the abdominal vena cava becomes compressed, decreasing venous return and causing CVP to demonstrate a negative correlation with IAP. The complex interactions between systemic vascular resistance, preload, afterload, and cardiac function can limit the clinical value of CVP measurements.[240] This review will focus on the definitions and clinical uses of CVP, the consequences of IAP on CVP measurement, the theory of abdominal zones, and methods of CVP measurement in the horse and other species.

5.2   Definition of Central Venous Pressure

Central venous pressure is cited as an indicator of the interaction between the cardiac pump and the vascular circuit returning the blood to the heart.[241-243] It is the mean vena cava or right atrial pressure, equivalent to right ventricular end diastolic pressure in the absence of tricuspid stenosis. Central venous pressure is measured in either the thoracic superior vena cava or the right atrium, and is determined by two factors.[244-247] One is cardiac function, including intrinsic factors such as heart rate, preload, afterload, contractility, as well as the function of the pulmonary circulation. The second is related to the return of blood to the heart, which is determined by the volume, compliance and resistance of the peripheral vascular system. [248] As right atrial pressure just prior to the onset of systole determines the preload of the right ventricle as well as cardiac output, CVP measurement has been utilized as a determinant of cardiac function and venous return to the heart.[249]

5.3   Measurement of Central Venous Pressure

Measurement of CVP in humans is commonly obtained from a central venous catheter placed in the internal jugular or subclavian vein, with the catheter tip placed in the superior vena cava just proximal to the right atrium. While the pressure obtained in the atrial chamber is the most accurate reading of CVP, presence of the catheter in the atrium risks dysrhythmias, atrial thrombosis or cardiac rupture.[173,250-252] CVP can also be obtained from the femoral vein, with the catheter tip placed in the inferior vena cava (IVC).[176,253,254] Correct CVP catheter placement can be determined by evaluation of the characteristic CVP waveforms noted by an electronic pressure transducer and monitoring system.[234]

Measurements are obtained at the end of expiration, with human patients in a supine position. The transducer is leveled at the approximation of the right atrium, taken to be the junction of the midaxillary line and the fourth intercostal space, called the phlebostatic axis.[255] This physiologic zero point is where the CVP is tightly regulated, and changes little with the patient's movement.[256-258] True CVP assumes that intra-thoracic and intrapericardial pressure are equal to atmospheric pressure.[253]

The normal range of pressure is between 1 and 7 mmHg in humans.[259] Superior vena cava pressures (SVCP) are approximately 0.08 mmHg lower than mean right atrial pressure, whereas inferior vena cava pressures (IVCP) are approximately 0.23 mmHg below the pressure in the right atrium.[260] In patients without ventilator assistance, the abdominal IVCP is comparable to mean right atrial pressure at end-expiration. FVP are less reliable as a measure of CVP; while mean differences are small, the limits of agreement are often wide.[176]

5.3.1 Measurement of Central Venous Pressure in Small Animals

Guyton initially investigated CVP in 38 anesthetized dogs in 1956. In these patients, he found the anatomic location of the physiologic zero point to be in the right ventricle just below the tricuspid valve.[261] This location produced minimal change in the measurements, whether the dog was rotated horizontally or vertically. Although this location is physiologically more accurate, small animal veterinary clinicians today use the level of the right atrium by convention to match the definitions in human medicine.[242]

In small animals, the CVP can be measured through jugular vein, femoral vein, or by saphenous central venous catheters. The tip of the catheter is expected to be level with the junction of the right atrium when using jugular catheters, and within the IVC when catheters are placed in the hind limb.[246,262] Positioning can be confirmed with radiography. Measurements of CVP can be obtained with a water manometer, or more easily and continuously with an electronic pressure transducer in sternal or lateral recumbency. Using an electronic transducer, the catheter is connected to a non-distensible extension set to prevent signal damping, and the pressure transducer is set level to the point equivalent to the right atrium. After calibration to room pressure, the catheter is connected and the waveform can be used to confirm and record the CVP waveform. In right lateral recumbency, the right atrium is level with the manubrium. In sternal recumbency, the zero-reference point is the scapulohumoral joint.

In dogs and cats, the normal reference range for CVP is between 0 and 5 H_2O, with values cited up to 10 cm H_2O.[244,247] Negative values for CVP are suggestive to be indicative of hypovolemia, and those greater than 12 to 15 cm H_2O can be indicative of

volume overload. Trends are likely more informative in clinical patients than values measured at a single timepoint. Similar to humans, CVP in small animals is cited as lowest during inspiration and higher during expiration. Ideally the CVP measurement is made it the end of expiration, at the lowest diastolic swing of the waveform in unassisted ventilation.[242] Conversely, patients provided positive pressure ventilation will have the highest CVP in inspiration, and lowest at the end of expiration. However, positive end-expiratory pressure ventilation patterns will increase CVP. As peripheral venous distention has no correlation to systemic arterial blood pressure, the clinician should regard peripheral pressure as inadequate to determine CVP and systemic vascular volumes.[242]

5.3.2   Measurement of Central Venous Pressure in Horses

Central venous pressure was first measured in the horse by Stephen Hale in 1733, which historically was also the first CVP recorded in any species.[263] Pressure was measured through the insertion of a glass tube into the jugular vein, and noted not only the pressure in the vessel, but the fact that the pressure was altered as the mare struggled against him. Central venous pressure is currently used in horses to estimate vascular volume and facilitate estimates of hydration and volume status.[264]

Measurement of CVP in the horse is often considered difficult by clinicians, due to the size of the animal and equipment available. Measurements have been obtained using commercial catheters (Intracath 16 gauge, Deseret Pharmaceutical Co., Sandy, UT; PICC Peripherally Inserted Central Catheter Set, 16 gauge, Arrow International, Reading, PA; Long Line Catheter LL1990, Mila International, Inc., Florence KY; 7 French, 110

cm catheter, Cook Veterinary Products, Indianapolis, IN) placed at the junction of the upper and middle third of the cervical jugular vein or in the mid-cervical region.[264-267] A disposable water manometer can be used to measure the pressure,[243,265,266,268] however, electronic piezo resistive sensor/transducers (Transpac IV, Hospira Inc., Lake Forest, FL) and monitoring devices (Datascope Passport LT, MAQUET Cardiovascular, Wayne, NJ; Medtronic Lifepak 12, Medtronic Physio-Control, Inc., Redmond, WA) have been validated.[264] The electronic monitors show improved repeatability, but may have inherent error from the power source, internal calibration, and the inherent error of the machine, between 1 and 2 mmHg.[264]

A second, more cost-effective method for measuring equine CVP involves a 3.5 French, 55 cm polypropylene urinary catheter inserted through a 14 gauge catheter.[269] While inexpensive, the length may not be appropriate for accurate CVP measurements; the catheter is rigid and may cause damage to the vascular endothelium. Serial measurements are also not possible, as the catheter cannot be left in place.

The external reference point has been cited as either the notch in the lateral tuberosity of the humerus (point of the shoulder) in standing horses or the manubrium of the sternum for horses in lateral recumbency, to approximate the level of the superior vena cava or the right atrium.[264-267] It was clearly noted that oscillations of the water manometer were observed when the catheter was in the right atrium, which provides confirmation of catheter placement in the heart.[265] Additional means to determine correct catheter placement include echocardiography, which in the horse requires specialized transducers (2.5 mHz) and computer programs with the ability to image the deep structures of the equine thorax.[265]

Measurement of CVP should be obtained with the manometer at the same level and the head in a neutral position at the withers for consistency in the standing horse.[270] Elevations in the head position will decrease the CVP, whereas lowering the head will raise the pressure. In humans, is stated that the pressure should also be obtained at the lowest oscillation due to increases in CVP caused during inspiration, referred to as the Kussmaul sign.[255,271] However, the oscillations in the horse have been shown to be <0.5 cm H_2O and are clinically insignificant.[270] A single measurement of CVP is likely adequate, as pressures obtained with a water manometer have <2% variability.[270]

Reference intervals for CVP have been established in both standing and recumbent, anesthetized healthy adult horses using a jugular catheter. In the standing horse, normal CVP values range from 7.5 +/- 0.9 H_2O to 12 +/- 0.6 cm H_2O.[243,266,268,270] However, comparisons of jugular pressures have not shown good concordance with right atrial pressures in standing horses.[265] As the tip of the catheter is withdrawn from the right atrium in 5 cm increments, the pressure measured will increase, and eventually these pressures overestimate the CVP. The recommended catheter insertion length is at least 40 cm to approximate the right atrial pressure in standing horses using a catheter placed in the mid-jugular vein in Quarter-type horses.[265]

Breed may affect catheter insertion site, as other studies have cited insertion lengths between 30 and 55 cm from the site of insertion to determine CVP in standing horses.[272] The horses in this study ranged from ponies to full sized animals, whereas the horses in the study by Wilsterman et. al. only measured pressures in American Quarter Horse mares between 454 and 520 kg.[265] While age, sex, breed, and type of horse did not

significantly affect CVP in the study Hall et. al., body weight did affect the measurements, with a correlation of 0.6 (P<0.001).[272]

In comparison to standing horses, jugular pressures in healthy horses under sevoflurane inhalant anesthesia did show a good correlation with pressures in the right atrium (r^2: 0.088; bias: 0.7 H_2O; LOA: -5 to 6 cm H_2O), which may be due to changes in systemic vascular resistance or body position.[267] Investigations into the effects of sedation and anesthesia on CVP, noted CVP significantly decreased after administration of vasodilatory medications, e.g. phenothiazine tranquilizers, whereas sedation with an alpha-2 agonist increased CVP in approximately half of the horses studied.[266] The change in CVP was attributed to the release of catecholamines and inhibition of postganglionic nerve conduction resulting in vasoconstriction and shifting of blood to the heart.[273-275] Alpha-2 agonists have also been shown to increase mean arterial pressure, diastolic pulmonary artery pressure, and carotid artery pressure.[276] After general anesthesia with the inhalant halothane, CVP values in lateral recumbency were higher than dorsal measurements, showing an effect of body position on CVP.[266] Pressures in lateral recumbency were elevated to between 20 and 30 cm H_2O, and should not be interpreted as evidence of fluid overload in anesthetized patients.[266]

5.4   Clinical Use of Central Venous Pressure Measurements

CVP must be interpreted alongside clinical signs to accurately understand the pressures measures and guide the course of therapy. Inaccuracies in pressure measurement can results from occlusions or kinks in the catheter, venous thrombosis, stenosis of the tricuspid valve, air gaps in the catheter or manometer line, inadvertent

catheterization of the right ventricle or pulmonary artery, and changes in the patient's position relative to the reference point.[242,277] Additional inaccuracies are related to positive end-expiratory pressure and chest wall compliance.

5.4.1   Central Venous Pressure Waveform

The CVP waveform is reflective of the various parts of the cardiac cycle, and when measured in the superior vena cava has defined components. The "a" wave is the first positive deflection and represents atrial contraction in normal sinus rhythm. Its deflection is dependent on the force of atrial contraction and the compliance of the right atrium, the right ventricle, and veins upstream.[277] The second positive deflection is the "c" wave that follows this "a" wave. It represents the bulge of the tricuspid valve into the right atrium during ventricular systole. It may also be related to the reflection of blood hitting the closed tricuspid valve in the right atrium.[277] The "x" descent is next, which represents the reduction in atrial pressure during ejection, followed by the "v" wave, which is the phase of rapid atrial filling when the tricuspid valve is closed. The height of the "v" wave is related to the amount of blood returning to the heart and the compliance of the right atrium and upstream vessels.[277] The "y" descent is the rapid ventricular filling phase in the final stage of the cycle. (Figure 5-1)

CVP should be measured at the base of the "c" wave or the "z" point, which is the pressure the right heart before onset of systole for estimation of preload. If this cannot be identified, the base of the "a" wave is a reasonable estimate. The CVP can also be timed, as it measured at the end of the QRS wave.[277] While CVP of <5 cm H_2O and >10 cm

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H_2O can sometimes be clinically assessed by visual assessment of jugular fill in humans, this parameter is inadequate, and has not been determined in veterinary species.[278]

5.4.2   Central Venous Pressure Measurement as a Resuscitation Endpoint

Internationally endorsed guidelines for the management of critically ill human patients recommend using CVP to assess cardiac preload and volume status, and as an endpoint for management of fluid resuscitation.[235,236] In the International Surviving Sepsis Campaign, central venous pressure remains one of the endpoints for early goal-directed therapy for patients with sepsis-induced tissue hypoperfusion.[236] The goal for resuscitation of severe sepsis and septic shock is a CVP of 8-12 mmHg within the first 6 hours of treatment. While the guidelines acknowledge limitations to CVP as a marker of intravascular volume and response to fluid administration, a low CVP is regarded as supportive of a need for fluid administration. It is also considered one of the most readily obtainable targets for monitoring resuscitation in the human ICU.[236]

5.4.3 Application of Central Venous Pressure in Small Animals

Uses for CVP in small animal include identification and monitoring of hypovolemia, similar to use in human patients.[262] In hypovolemia, CVP is low due to a loss of the effective circulating volume. Common causes in small animals include trauma, gastric dilatation and volvulus, and hemorrhage. Boluses of fluids (20 ml/kg crystalloids over 3 to 5 minutes) in resuscitation of the hypovolemic animal should increase CVP by 2 to 4 cm H_2O. If changes in CVP are not observed, the shock rate of fluid (60 to 90 ml/kg/hour) should be continued until CVP is 5 cm H_2O. In cases where continuous

monitoring is possible, the CVP should rise by 3 to 5 mmHg during a continuous bolus, and then continued fluid therapy is titrated until CVP is between 7 to 10 cm H_2O.[262] CVP is also commonly used in monitoring patients at risk for fluid overload, in particular cases with pre-existing heart disease or animals in renal failure undergoing diuresis. Smaller patients and neonates receiving high volume fluid therapy over 2-3 days are also likely to develop fluid overload should be monitored carefully. In dogs with gastric dilatation and volvulus, CVP has been used to monitor fluid resuscitation.[279]

5.4.4   Application of Central Venous Pressure in Horses

Measurement of CVP is more commonly used as a monitoring tool in research horses, rather than in clinical patients. Most reports in the literature are examples that are used to define the technique and its possible applications. Loss of intravascular volume secondary to dehydration through water deprivation and diuresis was noted to reduce the CVP in horses by 2.2 cm H_2O for every percentage point decrease in body weight.[270,280] Similarly, removal of a portion of the intravascular volume using controlled blood collection was also noted to decrease CVP in standing horses, causing CVP to fall below atmospheric pressure.[243] While the blood volume removed was modest (between 15 to 26% of total blood volume over one hour), the change in the circulating blood volume was observed by changes in CVP. The variability in CVP noted later in the study was attributed to activation of the autonomic nervous system and increased sympathetic tone, which may have increased venous tone and cardiac contractility and dampened the reductions in CVP caused by hemorrhage. CVP was determined to be a better indicator

of hypovolemia in an experimental model of blood loss than heart rate or biochemical indices, including lactate and blood gasses.[243]

Similar results were noted in a model of acute hemorrhage in rabbits, where CVP, blood flow and mean arterial pressure all fell significantly compared to baseline, whereas heart rate was unchanged.[281] Other studies in different species have failed to provide support for the use of CVP to measure changes in volume status.[238,282,283] While a negative value for CVP was consistently seen in animals with experimental blood loss, it would be impossible to determine if a CVP in the reference range was lower than baseline values in a clinical patient presenting with acute hemorrhage. The volume of blood or fluid lost could affect the CVP, or it could be altered by sympathetic tone, which increases in the face of hemorrhage. In cases of hemoabdomen, increased IAP could artificially elevate the CVP. Therefore, negative values for CVP may be useful for identification of hypovolemia in the face of acute hemorrhage, where as normal values are less diagnostic.[243]

In an experimental model of endotoxemia, CVP was also used to monitor circulating volume in horses.[284] An increase in cardiac output was noted after administration of lipopolysaccharide, with concurrent hypotension and decreased systemic vascular resistance typical of septic shock. CVP was significantly increased in horses receiving a 60 ml/kg bolus of isotonic fluids, whereas horses given a 15 ml/kg bolus showed a reduction in CVP. Administration of 15 ml/hg hypertonic saline and a hydroxyethyl starch did not note any change in CVP. While CVP was able to measure an increase in venous pressure, suggestive of increased venous return, it was not indicative

of, or influenced by, cardiac function, which may limit its use in monitoring fluid therapy in septic horses.

5.4.5 Critique of Central Venous Pressure for Measurement of Blood Volume

The belief that CVP is indicative of the status of the circulatory volume, and its usefulness as a guide for replacement therapy, has been called into question.[238,285] In fact, the initial paper that laid the groundwork for CVP cutoffs to guide goal directed therapy had no control arm to validate their statements.[64] Practice guidelines have stated that pulmonary edema is a common complication of excessive fluid resuscitation requiring careful monitoring of arterial oxygenation; therefore, use of a fluid resuscitation guideline that may harm the patient should be reevaluated.[286] Improved survival has also been shown in patients resuscitated to a low CVP, compared to those resuscitated to the higher guidelines of the Surviving Sepsis Campaign.[67] A meta-analysis of clinical trials that analyzed the relationship between CVP and blood volume, as well as studies that examined the usefulness of CVP in prediction of fluid responsiveness, showed little correlation between CVP and blood volume.[285,287,288] This is especially true in the presence of IAH, as CVP will also reflect the surrounding intra-thoracic pressure, demonstrating a positive correlation with IAP, and negative correlation with cardiac output.[74,237,238]

5.5 Relationship between Central Venous Pressure and Cardiac Preload

Preload is defined as the pressure causing the initial lengthening of the muscle before the onset of contraction. As stretch of the resting length of the muscle increases,

the peak tension during contraction increases as well, defined by the Frank Starling relationship. This premise states that the greater the resting diastolic volume, the greater the peak tension and stroke volume in relation to the ideal length.[78,289] Microscopically, this interdependence is related to an increase in overlap of myosin heads on active sites on the actin fibers,[78] as well as increased availability of ionized calcium through the spatial rearrangement of the myofilaments.[290,291] While the volume of blood returning to the ventricles can affect the initial loading conditions, the force of the contraction is related to the pressure produced by the myocardium and its muscle fibers.

While end diastolic volume can estimate cardiac preload and may assist in determining response to a fluid challenge, as a volume measurement it is not a true measure of force, and it is imprecise compared to measurement of pressure.[292] Echocardiographic measurements of cardiac volume are determined by measuring the radius of the chamber. As volume is related to the third power of the radius, small imprecisions in measurement of distance can result in large errors in volume estimates. In addition, the ventricular chamber dimensions are irregular and make accurate volume determination difficult. Finally, the maximum ventricular volume is limited by the pericardium, and cannot provide a linear relationship with volume in the cardiovascular system.[277] This supports the use of CVP as a more accurate assessment of preload.

In the normal heart, CVP is reflective of right atrial pressure, which is itself equal to the pressure within the right ventricle in diastole or the cardiac preload.[277] However, CVP is only indicative of preload at one timepoint in the cardiac cycle. As previously stated, the other components of CVP are not related to preload. In addition, the technical

aspects of determining the reference point of measurement complicate measurement of CVP.

5.6   Abdominal Zone Theory

The concept of interactions between IAP, IVCP, right atrial pressure, and venous flow has been described by the theory of abdominal vascular zone conditions. Similar to the physiology of the lung, the abdomen can be considered to consist of 3 vascular zones, which are dependent on the mean circulatory pressures, the pressure in the IVC, and the pressure in the abdominal cavity.[80,239]

A zone 3 abdomen describes the pressure and flow dynamics where the pressure within the IVC at the level of the diaphragm is higher than both abdominal pressure and the critical closing pressure of the vessel, defined as the pressure at which a vessel collapses.[80,239,293] In this situation, the abdomen acts as a capacitor, and increases in abdominal pressure can increase venous return to the heart as blood is compressed from the splanchnic vasculature and transferred to the IVC. However, if abdominal pressure is increased further to the extent that pressure in the IVC is lower than abdominal pressure, IVC venous return and flow is negatively correlated to increases in IAP. This is a referred to as a zone 2 abdomen, as the abdominal cavity serves as a collapsible Starling resistor. The change in pressure to cause this change in a canine model was very slim, averaging 1 mmHg.[80] In zone 1 conditions, there is no flow through the IVC due to compression of the inferior vena cava by abdominal pressures that exceed the critical closing pressure within the femoral vessel and the IVC.[239] Venous return ceases.

Experimental studies in dogs has shown that increases in abdominal pressure are known to increase cardiac output in zone 3 abdominal conditions, but decrease cardiac output if a zone 2 abdomen is present, due to alterations in both systemic venous return and changes in left ventricular preload.[80] Right ventricular preload will increase even with decreased venous return, due to increase in afterload caused by the transmission of abdominal pressure across the diaphragm to the thoracic cavity. As abdominal pressure increases, cardiac output will increase, initially, and then fall to baseline or even lower as pressures rise to 30 mmHg.[186,294] Pressure in both atria will rise, but remain less than the abdominal pressure. However, pressure in the IVC will rise corresponding to the abdominal pressure, creating a pressure gradient with the atria. Heart rate should not change, and MAP will show little difference with changing abdominal pressures up to 25 mmHg.[80]

Based on these findings, the IVC circulation is considered a two-compartment model, with the extra-abdominal component in the thorax and an upstream abdominal portion.[80] A vascular waterfall is formed at the level of the diaphragm. As the IVC return accounts for two-thirds of the systemic venous return, it is a major determinant of cardiac output.[295,296] Small increases in IAP, where right atrial pressure remains above abdominal pressure with a minimal gradient, will enhance venous return and increase preload with subsequent increases in cardiac output. If IAP exceeds right atrial pressure, a vascular waterfall forms, and the IVC forms an effective backpressure at the diaphragm, impeding venous return, and reducing preload and cardiac output. However, the abdominal zones only characterize venous return, not ventricular preload. Preload is a determined by both the venous return and cardiac function curves.[297]

Depression of the cardiac function curve is caused by decreased heart rate, decreased cardiac contractility, or increased afterload. Heart rate is not affected by increasing IAP, nor is contractility in some studies.[298] Pulmonary artery pressure can be increased by increasing abdominal pressures and reduced respiratory system compliance, resulting in increased right ventricular afterload.[299-301] Left ventricular afterload is relatively unchanged by IAP, as it is a combination of increased pressure in the abdominal arteries, which increases left ventricular afterload, and increased intra-thoracic pressure that reduces left ventricular afterload.[301-304]

The development of a vascular waterfall and a zone 2 abdomen is determined, in part, by the right atrial pressure, which is affected by intravascular volume status, cardiac function and pleural pressures. Changes in cardiac output and abdominal zone conditions can occur even if right atrial pressure is constant and high. However, hypervolemia may prevent vascular waterfall, especially in the face of IAH.[72,80] Changes in abdominal compliance, such as application of abdominal bandages, can reduce the amount of abdominal pressure that is required to result in a zone 2 abdomen and reduced cardiac output even with normal cardiac function.[305] Depressed cardiac function will increase the duration of a zone 3 abdomen, and cardiac output will then increase with increases in IAP. Improved cardiac output with abdominal binding will only occur if right atrial pressure is elevated, and the abdomen is in a zone 3 stage.[305] To predict the level of the abdominal zone, the magnitude of abdominal pressure, the intravascular volume status, and the baseline cardiac function must be considered to predict changes in cardiac output with changes in abdominal pressure.

Based on previous reports, it is not appropriate to estimate left ventricular preload with right sided pressures in the face of increased thoracic pressures.[240] Right-sided pressures do not reflect systemic venous return or the level of left ventricular preload. Simultaneous comparison of right atrial pressure and abdominal IVCP would be better to identify the abdominal zone status, left ventricular preload and the abdominal pressure that will maximize venous return and cardiac output.

Evidence for the vascular waterfall has been shown in canine models, where the upper abdominal inferior vena cava narrows with elevated IAP.[306-308] These initial studies believed that the obstruction occurred at, or just below, the diaphragm, termed the supra-hepatic vena cava. One theory was that the pressure in the abdomen elevates the diaphragm and narrows the IVC foramen as it approaches an oblique angle with the cross-sectional area.[306,308] Others proposed that the change in pressure from the abdominal to thoracic cavity causes collapse of the supra-hepatic IVC due to changes in the critical closing pressure of the vessel.[307] More recently, computed tomography studies noted the compression was actually in the upper intrahepatic segment, not the supra-hepatic portion of the vessel.[309] This section may be susceptible to narrowing, as it also known to collapse during normal respiration.[310] These findings may also explain the lack of hepatic venous outflow obstruction noted in patients with IAH.[19] The lack of compression of the supra-hepatic IVC is also consistent with Starling's resistor model, which demonstrate that the functional narrowing of a tube at a site of a change in pressure is difficult to elicit in tubes less than 3.5 cm long.[311] The supra-hepatic vena cava is very short, and may resist changes in pressure.[309]

5.7   Correlation between Central Venous Pressure and Intra-Abdominal Pressure

Decreases in CVP can reflect hypovolemia or low cardiac output, and distributive shock with loss of tone in the peripheral vasculature. In contrast, elevations in CVP are associated with a reduction in cardiac contractility, and expanded blood volume causing high venous return.[248] An additional cause for increased CVP is related to increased intra-thoracic pressure. While thoracic pressure can be elevated in the face of positive pressure ventilation, thoracic pressure could also rise secondary to an increase in abdominal pressure caused by abdominal effusions due to transmission of IAP across the diaphragm. In addition, intra-abdominal insufflation with gas will also cause a corresponding increase in measured CVP.[75,186,312]

Early observations comparing CVP to IAP measurements during laparoscopy noted that IAP up to 25 mmHg produced a similar increase in CVP by about 1.3 times that of the insufflated pressure in humans.[186] Pulse rate and mean arterial blood pressure also increased, and an increase in cardiac output was observed. Similar findings were noted in pigs where CVP rose with increases in IAP, however, mean arterial pressure (MAP) in these animals was not significantly affected. [42,101]

Interestingly, others have shown a more complicated correlation, with a positive correlation at IAP less than 15.7 mmHg, and then a negative correlation as IAP continued to increase. This inverted U shaped curve was proposed to be related to the disease process, as pancreatitis is noted to cause severe capillary leak and reductions in circulating volume caused by release of inflammatory mediators.[313] However, the changes in CVP correlation may be more indicative of the conversion to a zone 2 abdomen, where pressure in the IVC is lower than abdominal pressure, and IVC venous

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return and flow is negatively correlated to increases in IAP.[72] Similar findings were noted in burn patients, where vital signs were poorly predictive of survival, whereas hemodynamic measurements including CVP, cardiac index, stroke volume, systemic vascular resistance and oxygen transport measurements were more indicative for guiding therapy and response.[314]

Additional papers have supported the belief that CVP is a poor predictor of preload, especially in the resuscitation of major burn injury [314-318] Based on one report, it is likely influenced by abdominal pressure more than volume status of the patient, and may lead to an underestimation of the fluid requirements. A high CVP may be supportive of ACS, and should prompt measurement of IAP. Although CVP alone is not reflective of blood volume, or even useful as a guide for fluid resuscitation, it can be used combined with other indices, including urine output, MAP, heart rate, hematocrit, and venous oxygen saturation for a more complete picture of cardiovascular status.[313]

In humans with naturally occurring IAH (>15 mmHg), the relationship between pressure in the superior vena cava and IVC is directed by the intra-vesicular pressure relative to the pressure in the IVC. A large abdominal pressure causes the waterfall effect, and IVC pressure will not reflect SVC pressure. [80,187] Although pressures in the IVC can reflect IAP as it increases, it is not strongly correlated with IAP at lower pressures. However, this study suggests that at higher IAP, CVP may be useful as a surrogate measure of IAP.[187]

5.7.1   Inferior Vena Cava Pressure as a Measure of Central Venous Pressure

IVC cannulation is often selected to measure CVP, as the catheterization site has fewer risks of complications than one placed in the SVP.[254] The IVC is also highly compliant, and it size and diameter vary in concert with changes in CVP and volume, and changes in intra-thoracic and intra-abdominal pressure.[259,319] Forward flow into the right atrium is biphasic, and is highest in ventricular systole. Typically, pressure and volume are reciprocal in the IVC, in that increase in pressure will decrease flow and vice versa. For example, when intra-thoracic pressure falls during inspiration, vena cava pressure should decrease and flow increase. The IVC therefore acts as a capacitance resistor. At low IAP, systolic flow is greater than diastolic flow, and as pressure increases, forward flow is reduced, as well as venous collapse, followed by IVC dilation at high pressures.

In dogs under general anesthesia, Berg et. al. noted an agreement of 2 mmHg between IVCP and CVP measured in the superior vena cava and abdominal IVC, with a mean difference of 0 to 0.1 mmHg at end-expiration during both spontaneous and mechanical ventilation. These dogs were subjected both to volume depletion, and upper airway obstruction.[320] Harman et. al. observed that increasing IAP to 40 mmHg with an air-filled intra-abdominal bag in dogs increased pressure in the IVC to level similar to the IAP. Lacey et. al. showed similar results in rabbits, however pressures in the superior vena cava and femoral artery were not good estimates of IAP.[133]

Comparisons of the differences between SVCP and IVCP in humans noted an increase in IVCP and SVCP as IAP increased.[132] In pediatric patients, a right atrial catheter was shown to correlate well with an IVC catheter for both mechanically ventilation patients and those spontaneously ventilating.[253] Joynt et. al. observed similar findings, in that IVCP and SVCP correlated well, regardless of IAH.[254] IVCP is reflected

from the right atrium through the inferior vena cava, and has been shown to reliably predict IVCP in multiple positions along its course in adults and children [174-176,253,254,260,320-323] This suggests that IVC pressures may be valid as a measure of CVP.

5.7.2   Ultrasonographic Evaluation of Venous Diameter and Flow

Ultrasonographic evaluation of the vasculature is an effective method for determining venous diameter and flow in real time in patients with altered cardiovascular parameters. The IVC has been shown to change in both size and shape with changes in CVP and intravascular volume.[259] Sonographic measurement of this vessel has been shown to be an effective and noninvasive tool for measuring CVP.[319] IVC diameter can also be altered by inspirations that decrease intra-thoracic pressure and increase IAP.[324] Normally, at low pressures, the IVC is collapsed at peak inspiration, as the pressure in the thorax is negative. If intra-thoracic pressure increases, the IVC remains open and venous return is solely dependent on the pressure gradient between the venous system and the right atrium. IVC diameter also decreases in ventricular systole and by changes in body position.[259,324] There is some evidence that IVC diameter could be used to estimate CVP or right atrial pressure in spontaneously ventilating patients as positive correlations were noted between IVC size and CVP or right atrial pressure.[259] All studies evaluated noted correlation between IVC and direct invasive measurements of CVP.

Ultrasonography of the femoral arteries and veins has been used to assess diameter and flow to monitor changes that may occur during periods of increased IAP, as in laparoscopic surgery.[325-327] Initial work noted a decrease in peak velocity in the common femoral vein of patients insufflated to 14 mmHg for laparoscopic

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cholecystectomy.[326] A femoral vein catheter noted an increase in venous pressure after insufflation, although cross-sectional area was not affected. Millard et. al. noted similar findings in their study that observed that peak velocity was reduced by 42% in the common femoral vein after insufflation to 13 to 15 mmHg in a reverse Trendelenberg position.[327] A duplex scanner obtained venous flow from the velocity and square of the vein radius multiplied times π. Contrary to previous work, mean cross sectional area of the common femoral vein in Sobolewski's study was noted increase after insufflation of the abdomen to between 14 to 16 mmHg, whereas venous flow decreased.[325] Interestingly, deflation of the abdomen did not return femoral vein diameter to its pre-surgical diameter, but venous flow rebounded to levels similar to baseline.

5.7.3   Comparison of Equine Intra-Abdominal Pressure and Central Venous Pressure

Central venous pressures measured in the right atrium were observed in an equine laparoscopy model to have no correlation to IAP measured from the abdomen by the laparoscopic insufflator. Although right atrial pressures increased, this change was temporally associated with sedation with a bolus of detomidine, and not with insufflation of the abdomen.[328] Similar findings showing this lack of correlation were noted in a study that treated horses with a constant rate infusion of detomidine, however, no alterations in right atrial pressure were observed secondary to the sedation, which was given continuously over time.[223] In dorsal recumbency under anesthesia, pressures in the right atrium did increase with insufflation, and were similar in magnitude to IAP, but correlations were not determined.[329] In each study, the abdomen was insufflated to 15 mmHg. In a case report of naturally occurring IAH in the horse, CVP was elevated along

with increased IAP, but correlations could not be obtained from the data available from the two horses.[10] These limited examples in the literature provide evidence that CVP may be correlated with IAP, and support additional investigations to determine the effects of the waterfall phenomena in horses.

Figure 5-1 Central venous pressure (CVP) waveform, relative to the electrocardiogram (ECG). The wave labeled a in the CVP waveform represents atrial contractions, noted as the P wave in the ECG. The c wave in the CVP waveform represents the bulge of the tricuspid valve during ventricular systole, represented by the R wave in the ECG, composed of a negative deflection (Q) a positive deflection (R), and a second negative deflection (S). The x decent for the c wave in the CVP waveform represents the loss of atrial pressure during ejection, followed by the v wave, caused by rapid atrial filling after the tricuspid valve closes, noted by the T wave in the ECG. The y decent in the CVP waveform is the rapid ventricular filling stage after tricuspid valve opening. **Adapted from Magder, S.** Understanding central venous pressure: not a preload index? *Curr Opin Crit Care* 2015;21:369-375.
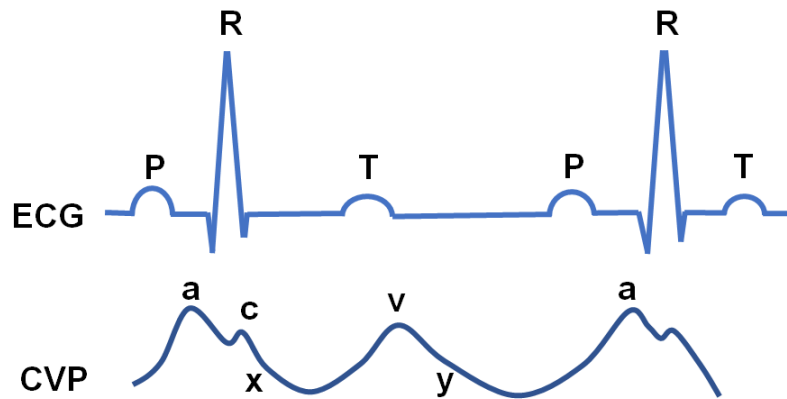
Chapter 6

Abstract

Central venous pressure (CVP) is the pressure in the right atrium or vena cava at the level of the atrium. Previous reports have shown that CVP can be either misleading or lack correlation with hemodynamic function. One explanation could be the fact that intra-abdominal pressures (IAP) and intra-thoracic pressure gradients are not often assessed during the recording of CVP. As such, resuscitation to absolute measurements of CVP in patients with intra-abdominal hypertension (IAH) could cause under-resuscitation, inappropriate diuresis, or poor end organ perfusion. The purpose of this study was to evaluate the effect of increased IAP on CVP, mean arterial pressure (MAP), femoral venous pressure (FVP) and femoral vein diameter and blood flow in the horse. Horses were subjected to 6 stepwise elevations in IAP created using insufflation of carbon dioxide gas (CO_2). Vital parameters and hemodynamic parameters were assessed at each pressure after an equilibration period. Horses were also subjected to a sustained period of IAH (20 mmHg for 30 minutes) with measurements taken every 5 minutes. Results found that CVP was not significantly altered by IAP, and did not correlate with IAP measurements, but demonstrated a biphasic trend. Femoral pressure was

significantly correlated with IAP, and was more accurate during the steady state phase of IAH than an intra-peritoneal piezo electric microsensor catheter. IAP did not alter femoral vein diameter or blood flow, and vital parameters were unchanged during the experiment. These results indicate that FVP may be useful to monitor changes in IAP and could be used clinically to observe trends in horses at risk for IAH.

6.1   Introduction

Central venous pressure (CVP) is cited as an indicator of the interaction between the cardiac pump and the vascular circuit returning the blood to the heart.[241-243,280] It is measured either in the thoracic superior vena cava or the right atrium,[265,266] and is determined by two factors. One is cardiac function, including intrinsic factors such as heart rate, preload, afterload, contractility, as well as the function of the pulmonary circulation. The second is related to vascular return, which is determined by the volume, compliance and resistance of the peripheral vascular system as well as the CVP.[248] As right atrial pressure just prior to the onset of systole determines the preload of the right ventricle as well as cardiac output, CVP measurement has been utilized as a determinant of cardiac function and venous return to the heart.[249]

Normal CVP in the standing horse is between 7.5 and 12 cm H_2O, whereas the pressure in horses in recumbency can be much higher, up to 31 cm H_2O.[243,266,267,270,272] Decreases in measurements of CVP can occur related to hypovolemia, increased venous resistance, or secondary to a hyperdynamic heart.[248] Loss of intravascular volume secondary to dehydration through water deprivation and diuresis was noted to reduce the CVP in horses by 2.2 cm H_2O for every percentage point decrease in body weight.[280] Similarly, removal of a moderate portion of the intravascular volume using controlled blood collection was also noted to decrease CVP in standing horses, causing CVP to fall below atmospheric pressure.[243]

In contrast to reductions in CVP, elevations in measurements of CVP are associated with a reduction in cardiac contractility, expanded blood volume causing high venous return, and distributive shock with loss of tone in the peripheral vasculature.[248]

An additional cause for increased CVP is related to increased intra-thoracic or intra-abdominal pressure (IAP). While thoracic pressure can increase in the face of positive pressure ventilation, iatrogenic reasons could include laparoscopic insufflation for thoracoscopy.[330-332] Thoracic pressure could also rise secondary to an increase in abdominal pressure, as the pressure within the thorax reflects not only pleural, but also IAP.[75,85,86,312]

Internationally endorsed guidelines for the management of human critically ill patients recommend using CVP measurements as an endpoint for management of fluid resuscitation for early goal-directed therapy.[236] It is believed that CVP accurately reflects intravascular volume, and both texts and review articles state that a low CVP is associated with volume depletion and a high CVP indicates the patient is volume overloaded. The belief that CVP is indicative of circulatory volume status, and its usefulness as a guide for replacement therapy, has been called into question.[285] Clinical trials analyzing the relationship between CVP and blood volume, and studies that examined the usefulness of CVP in prediction of fluid responsiveness, showed little correlation between CVP and blood volume.[285,287,288] Improved survival was noted in patients resuscitated to a lower CVP than the recommended guidelines, as CVP performed poorly in monitoring fluid balance.[67] Excessive fluid supplementation can result in complications including pulmonary edema and hypoxia, and the use of absolute values of CVP as a guide for fluid responsiveness may cause patients harm.[286]

While increased IAP are known to elevate both intra-thoracic pressures and CVP, the relationship between CVP and IAP is complicated. As pressure rises in the abdomen, blood flow in the inferior vena cava (IVC) may increase as the pressure moves blood

from the splanchnic organs to the central circulation. As long as the pressure within the abdominal portion of the IVC is above that of the critical closing pressure, referred to as a zone 3 abdomen, the abdominal cavity acts as a capacitor, and blood flow to the heart will increase.[80,239,293] As abdominal pressure continues to climb, the IVC becomes compressed and blood flow is gradually reduced from the abdomen and hind limbs. This is referred to as a zone 2 abdomen, and the abdomen cavity is described a serving as a Starling resistor.[80] Once closing pressure is exceeded, called a zone 1 abdomen, blood flow will cease.[239]

This parabolic correlation between CVP and abdominal pressure has been observed in humans, where CVP initially demonstrated a positive correlation, followed by a negative correlation as pressure in the abdomen continued to increase.[313] Significant differences between blood flow in the superior vena cava and inferior vena cava have also been shown with increased IAP, supporting the description of a vascular waterfall and zone 2 abdomen in patients with IAH.[187] The correlation between CVP and IAP has been identified in both human patients and laboratory animal models.[92,101,186,318] However, there few reports of the effects of increased IAP on CVP measurements in horses. Increases have been described, relative to standing CVP measurements, in healthy horses under anesthesia, but were assumed to be associated with changes in body position.[266] The abdominal pressure in this study was not defined. In a case report of two horses with IAH secondary to peritonitis, CVP was also increased, but was only measured at one time point in the disease.[10] While right atrial pressure was not affected in horses insufflated experimentally with CO_2 gas to pressures of 15 mmHg, the effects of a range of pressures, or the higher pressures seen in cases of intra-abdominal hypertension

have not been investigated in horses.[223,328] In addition, the direct effects of increased IAP on CVP measurements in the horse have not been described.

The purpose of this study was to further examine the relationship between IAP and venous pressures in healthy horses. We expected that as pressure increased, the abdomen would first respond as a capacitor, increasing CVP measured, followed by a reduction in CVP as the abdomen transitioned to a zone 2. Our first hypothesis was that CVP would show a positive correlation to IAP at low pressures, but would become negatively correlated at higher levels. If a relationship between CVP and IAP was derived, the second goal of this study was to evaluate the role of CVP as a predictive measure of IAH. The hypothesis was that as pressures within the abdomen increase, the CVP measured would correlate with the changes in abdominal pressure as an indirect measure of IAH. Third, as pressures in the abdominal compartment may affect pressures in the vasculature of the hind limbs, the hypothesis was that pressure and flow in the femoral vein would correlate with the pressure in the abdomen for venous return from the limb. Assessments of vascular hemodynamics of the femoral vessel included venous pressure, flow and venous diameter. Our final hypothesis was that as femoral pressure increased, flow would decrease and venous diameter would increase with increasing IAP.

6.2   Materials and Methods

6.2.1   Animal Selection and Instrumentation

Seven university owned horses were included in the study, selected as a convenience sample from the teaching herd. They were considered clinically healthy

based on a physical exam, thoracic and abdominal transcutaneous ultrasound, and transrectal evaluation. Horses were excluded if there was a medical history of colic in the previous 6 months, previous abdominal surgery, ongoing pregnancy, or a body condition score greater than 8 or less than 2.[333] The Auburn University Animal Care and Use Committee (Protocol #2015-2670) approved this study. The horses were held off feed, but not water, for 12 hours prior to instrumentation to reduce variability in gastrointestinal fill.[11,12]

The horses were restrained in standing stocks and a handler maintained the head in a neutral position at the level of the withers during the experiment. The horses were sedated with a bolus of detomidine hydrochloride (6 micrograms/kg) for placement of the catheters and cannulas. Sedation was maintained throughout the experiment with detomidine at a constant rate infusion of 0.8 micrograms/kg/min, reducing the rate by half every 15 minutes thereafter, as needed. All insertion sites for both cannulas and catheters were clipped and sterilely prepared.

For catheter placement in the jugular veins, a subcutaneous bleb of 2% mepivacaine was performed (0.5 to 1 mL) to desensitize the skin over both the right and left jugular vein. A 14-gauge polytef catheter was placed in the left jugular vein in the proximal third of the neck for administration of sedation. A 5 French, single lumen, 60 cm central venous catheter was placed in the right jugular vein, in the approximate midpoint of the cervical jugular vein, using the manufacturer's provided trocar and peel-away introducer for monitoring the CVP (Peripherally Inserted Central Catheter, silicone catheter kit, Mila International, Inc., Florence, KY). The tip of the catheter was confirmed to reside in the thoracic vena cava, as verified by oscillations in the CVP

tracing corresponding to respirations.[266] The CVP measurement was performed at the end of expiration, and the reference point was set at the notch in the greater tubercle at the point of the shoulder. An electronic monitor (Abvisor Vital Signs Monitor, Surgivet USA, Smiths Medical, Dublin, OH) was used to record continuous pressure readings, connected to the catheter by a disposable pressure transducer (TransStar Pressure Monitoring System, Smiths Medical, Dublin, OH).

To obtain femoral venous pressures (FVP), a 14 gauge, 30 cm, polyurethane single lumen catheter (Central Venous Catheterization Set, Arrow International, Inc., Reading, PA) was inserted into the vessel on the medial aspect of the hindlimb, at a level two-thirds of the distance from the stifle to the hock. The skin and subcutaneous tissues were anesthetized with 1 cm of 2% mepivicaine, and the catheter was placed using a standard technique from distal to proximal into the femoral vein. The catheter was secured to the skin with 2-0 polypropylene suture and a tape butterfly, and the catheter flushed with heparinized saline. The femoral catheter was connected by pressure tubing and a second disposable electronic transducer to the electronic monitor to obtain measurements of FVP. The reference point was identical to that used for CVP. Placement of femoral catheters was randomly alternated between the right and left hindlimb for each horse.

An arterial catheter was placed in the transverse facial artery for direct blood pressure measurement. The skin and subcutaneous tissues were anaesthetized with 0.5 to 1 ml of 2% mepivacaine. A 20 gauge, over the needle, polytef catheter was placed in the vessel, and secured to the skin with cyanoacrylate adhesive. An extension set and pressure tubing connected the artery to a third disposable pressure transducer and the

electronic monitor. The reference point for direct arterial pressure measurement was identical to that for the central venous and femoral catheters.

For placement of abdominal cannulas, the subcutaneous skin and underlying muscle were anesthetized with approximately 3 to 5 ml of 2% mepivacaine. A stab incision was performed through the skin using a #15 blade, and the trocarization cannula was bluntly introduced into the abdomen, noted by the audible release of air into the abdomen. The insertion trocar was removed and a commercial piezo electric microsensor strain-gauge (Codman Microsensor ICP Transducer, DePuy Synthes, Raynham, MA) attached to a 100 cm nylon catheter was placed into the abdomen to a total distance of 10 cm, after subtracting the width of the body wall (measured during the trans-abdominal ultrasound evaluation). The catheter was secured with white medical tape after removal of the insertion cannula. The microsensor catheter was attached to an electronic monitor (Codman Microsensor Transducer Monitor, DePuy Synthes, Raynham, MA) for direct measurement of IAP from the peritoneal cavity. A second trocarization cannula was placed in the left flank, level with the ventral aspect of the tuber coxae using a similar technique. After the stylet was removed, the cannula was left in place, and attached to a medical laparoscopic insufflator (Stryker 10 L High Flow Insufflator, model F20-342200, Stryker, Kalamazoo, MI) for introduction of sterile CO_2 gas.

6.2.2   Measurement of Pressure and Vascular Hemodynamics

After placement of the catheters and cannulas, the horses were allowed to equilibrate for 30 minutes and then baseline measurements were obtained for CVP, direct IAP, FVP, and vital parameters including heart rate and respiratory rate. Pressure

measurements were obtained in triplicate at all time points. After 5 minutes, a second set of measurements were obtained to evaluate the horses at an insufflation pressure of 0 mmHg. The horses were then insufflated to 8 mmHg using the laparoscopic insufflator and maintained at this pressure for 5 minutes to allow for equilibration. All measurements were repeated. The horses were then incrementally insufflated in a similar manner to 10 mmHg, 12 mmHg, 15 mmHg and 20 mmHg of IAP, with measurements obtained in a similar manner for all parameters. Once horses reached an insufflation pressure of 20 mmHg, they were maintained at this pressure for 30 minutes, with measurement of CVP, direct IAP, femoral pressures and vital parameters every 5 minutes. After 30 minutes at steady state, the horses were allowed to passively decompress through the left-sided cannula, and all instrumentation was removed.

Measurements of femoral vascular hemodynamics were obtained using a variable frequency annular phased array transducer (S3-1 probe, Philips North America Corp., Andover, MA) with a 3 to 21 MHz extended frequency array and color Doppler analysis using an ultrasound machine (Philips HD 11XE, Philips North America Corp., Andover, MA). The probe was placed perpendicular to the skin, in the hind limb contralateral to the limb being used for femoral pressure monitoring. The probe was positioned 2 cm proximal to the common femoral vein bifurcation, medial and proximal to the stifle joint. The skin was prepared with alcohol for transduction of the signal. Measurements of femoral vein diameter, peak averaged velocity, time averaged velocity, femoral vein area, and flow were obtained at 5-minute intervals starting at baseline, time 0. Vessel diameter was measured from two-dimensional images frozen in real time, and flow velocity was analyzed with the commercial software provided by the machine. Time-averaged mean

velocity and volumetric flow were determined from the femoral venous waveform over the length of one cardiac cycle.

6.2.3   Statistical Analysis

Data was collated and collected using commercial software (Excel, Microsoft Windows 2010, Microsoft, Inc., Redmond, WA). All pressure measurements were obtained in triplicate and were averaged for each timepoint and measurement for statistical comparison. Results are reported as median for integers, or mean and standard deviation. Homoscedasticity was evaluated using a Bartlett's test for comparison of data, and visual inspection of boxplots of the data. For data with normal distributions, comparisons were performed with a one-way analysis of variance, and significant differences were further analyzed with post hoc testing using Tukey's HSD. A Kruskal-Wallis rank sum test was performed for non-parametric comparisons of medians, including data such as heart rate and respiratory rate. A Nemenyi's test of multiple comparisons for independent samples was used for significant comparisons, which also corrects for inflation of type 1 error. A value of $P<0.05$ was considered significant.

Inter-rater reliability was obtained to determine the concordance between different methods of pressure measurement. A mixed procedure analysis was used with the Shrout-Fleiss interclass correlation (ICC) for inter-rater reliability to determine the concordance between different methods of pressure measurement. Statistical analysis was performed using commercial statistical software (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing,

Vienna, Austria. URL https://www.R-project.org/; SAS 9.4 Language Reference Concepts, SAS Institute, Cary, NC).

6.3 Results

6.3.1 Signalment

Seven adult horses were obtained for the study, including two mares and five geldings. The breeds selected included five American Quarter Horses, and a single example each of an American Warmblood, a Tennessee Walking Horse, and a Thoroughbred. Ages ranged from 8 to 25 years, with a median age of 15 years. Horses ranged in weight from 445.0 to 596.3 kg, with an average weight of 540.7 kg. Body condition scores, on a scale from 1 to 9, were between 5 and 7.5.[333] (Table 6-1)

6.3.2 Complications

The catheter placement and technique for CVP, FVP and MAP measurement was routine. Placement of the cannulas for IAP measurement and insufflation was straightforward, and no significant complications were observed in any horse. Mild subcutaneous emphysema was observed at the sites of cannula introduction in both flanks, which resolved within 12 hours.

6.3.3 Vital Parameters

Measurements of heart rate showed no difference in variance for measurements obtained at each timepoint during the experiment (P=0.9989). These measurements also

showed no specific trend over time, and did not significantly differ over the course of the experiment (P=0.3985). (Figure 6-1) Respiratory rate, however, tended to increase as pressure in the abdomen increased (P=0.0009439), but post-hoc comparisons using a Nemenyi analysis were not significantly different (P>0.1197). (Figure 6-2)

6.3.4 Arterial Blood Pressure Measurement

Measurements of arterial blood pressure demonstrated a trend for an increasing MAP pressure as pressure in the abdomen increased above baseline (P=0.02887). (Figure 6-3) However, post hoc analysis using a Nemenyi analysis showed that comparisons were not significantly different (P>0.13).

6.3.5 Central Venous Pressure Measurement

CVP were obtained in all horses for all timepoints. The variance between measurements obtained at each timepoint during the experiment was not significantly different for systolic, diastolic, or mean values for CVP (P>0.6884). (Figures 6-4, 6-5, 6-6) The CVP tended to trend upward as pressures increased to 12 mmHg, but then declined as pressures increased further. Differences in mean, systolic and diastolic central venous pressure were not significant over time (P>0.06846), although changes in systolic pressure did approach significance. After pressures reached 20 mmHg at time 30 minutes, central venous pressures trended down for systolic, diastolic, and mean measurements of pressure, and appeared to stabilize at a lower pressure, although pressures were not significantly different at steady state (P>0.8333).

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Comparisons of CVP were performed with insufflation pressures, as well as direct pressures measured from the abdomen with the microsensor catheter. Scatterplots noted no pattern of distribution for the observations for mean, systolic or diastolic measures of CVP. The inter-rater reliability test noted little consistency between the measurements over the course of the experiment (ICC<0.47706). In the steady state phase, where pressures were held constant at 20 mmHg, the consistency between methods of measurement fell further (ICC<0.020148).

6.3.6 Venous Hemodynamics

Venous diameter was not changed over the course of the experiment, despite increasing pressures in the abdomen (P=0.9963). (Figure 6-7) Comparisons with insufflation pressures as well as pressures measured from the abdominal cavity with the microsensor catheter noted no correlation between abdominal pressures and venous diameter (P<0.48033). Peak average velocity as well as time averaged velocity in the femoral vein were also consistent, and demonstrated no change with changes in IAP (P=0.9993 and P=0.9306, respectively). (Figures 6-8 and 6-9)

The FVP were significantly increased from baseline as pressures in the abdomen increased from 0 mmHg, from time 10 minutes through 60 minutes (P<2.2e-16). (Figure 6-10) Pressures were significantly different from baseline as pressure in the abdomen reached 5 mmHg (time 10 minutes). As pressures continued to rise, venous pressures followed in a similar step-wise manner, until reaching 20 mmHg. Femoral venous pressures remained consistent until the end of the study, with no significant variation at the steady state pressure (P=0.9428).

The inter-rater reliability between the three methods of measurement noted a high consistency between FVP, direct pressure measurement from the abdomen, and the pressure instilled by the laparoscopic insufflator (ICC=0.92). However, at steady state the consistency between methods of pressure measurement was more variable (ICC=0.24). Comparisons between insufflation pressures measured from the abdomen with FVP were more consistent (ICC=0.47).

A Bland-Altman plot was used to evaluate the measured mean FVP, with consideration for repeated measures.[334] Direct pressure measurement from the abdomen tended to overestimate the insufflation pressures, suggestive of a systematic error, with a mean bias of 1.9 mmHg and limits of agreement between -2.3 mmHg and 6.1 mmHg. (Figure 6-11) FVP overestimated direct abdominal pressures to a greater degree (bias: 13.8 mmHg; limits of agreement: 6.5 mmHg, 21.1 mmHg), (Figure 6-12) with similar results noted when mean femoral pressure was compared to the insufflation pressures (bias: 15.7 mmHg; limits of agreement: 7.4 mmHg, 24.1 mmHg). (Figure 6-13) The limits of agreement were wide for both comparisons.

6.4   Discussion

The purpose of this study was to determine the correlation between CVP measurements and the pressure within the abdominal cavity, and to assess the effect of increasing IAP on venous hemodynamics. To our knowledge, this report is the first to investigate the correlation between IAP and CVP in the horse. Although the data was unable to demonstrate significant differences between groups due to the high variance of CVP measurement at each timepoint, a trend observed that CVP increased with IAP up to

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a pressure of 12 mmHg, followed by a decrease in CVP as IAP rose to 15 mmHg, and then 20 mmHg. As the abdomen in other species has demonstrated a biphasic response of CVP to increases in IAP,[88,313] it suggests there is a cut off, around 15 mmHg in these horses, where IAP may negatively affect venous return in the horse. It also suggests that CVP may not be a reliable indicator of venous return and intra-vascular volume when pressures in the abdomen are high.[335,336] Further investigations would be warranted to further define and describe the abdominal zone theory in horse, to help establish guidelines for therapy of IAH. Post hoc statistical analysis suggests that a total of 33 horses would be needed to provide adequate numbers for statistical comparison (power: 0.9; type I error rate 5%).

Unlike CVP, pressure in the femoral vein demonstrated an excellent correlation with pressures measured directly from the abdomen with both the microsensor catheter, as well as the abdominal insufflator.[337] Similar results were noted in humans insufflated for laparoscopic surgery, supporting the use of FVP as a substitute for direct measurement of IAP in horses.[88] The mechanism for this observation relates to the abdominal zone theory; as pressure increases in the zone 3 abdomen, blood is forced forward out of the splanchnic vasculature, but the rising pressure prevents venous return of the limbs until pressure rise above that in the abdomen. This continues until a zone 2 abdomen begins to compress the inferior vena cava, resulting in decreased venous return, progressive storage of blood in the limbs, edema, and thrombosis of the vasculature.[88] It is suggested that the close parallels of FVP and IAP are due to the lack of anastomoses that could bypass the inferior vena cava and return blood to the heart.[88] Based on the

results of this study, horses appear to have similar femoral vein hemodynamics in the face of increasing IAP.

Pressure was more variable between the methods of measurement at steady state, where the pressure was held constant at 20 mmHg. However, the pressure measured from the femoral vein showed improved accuracy when compared to the laparoscopic insufflator than the direct pressures measured from the microsensor catheter. The poorer correlation of measurement from the microsensor catheter with the insufflator may have been partially due to the increased variance this catheter can display as it cannot be calibrated in vitro, which could result in drift of the measurements over time.[155] In addition, there is a slight time delay in pressure measurements with the microsensor catheter that may allow pressure to be skewed by short-lived changes such as body position, coughing, urinations and defecations that were noted to briefly spike IAP in the horses in this study. Finally, the accuracy of the electronic system is stated by the manufacturer to be within +/- 1 mmHg or 1% of the measurements, which could account for a portion of the differences noted from the insufflation pressures for both femoral pressures measured with the electronic system and microsensor measurements obtained by a separate monitor. Although both systems correlated well, FVP may provide improved prediction of IAP trends at high levels of IAP than the microsensor catheter, which has only been validated in horses without IAH.[16]

While correlation between the methods of measurement were excellent, the bias and limits of agreement for the femoral catheter were large. The WSACS has proposed that for a validation of a novel technique, pressure measurements must demonstrate a bias of <1 mmHg and precision of 2 mmHg, or limits of agreement (LOA) between -4 and 4

mmHg for IAP techniques to be interchangeable using a Bland-Altman analysis.[118] The microsensor catheter demonstrated a bias slightly above the cutoff, whereas its precision was acceptable based on the guidelines and the variation due to the accuracy of the machine. However, the femoral venous catheter significantly overestimated the IAP, with limits of agreement approximately twice that of the microsensor. The wide limits of agreement could be due partially to the small sample size.

An explanation for the large bias noted in FVP is the distal insertion site in the limb, which measures pressure in the vessel below the level of the abdomen. Pressures at this site, which averaged 14.1 +/- 5.3 mmHg at baseline, indicate that pressure in the standing horse are higher in the distal limb to allow for venous return against gravity. Extremity blood pressure is also much higher in other species, and in humans, blood pressure has been observed to increase up to 65 mmHg higher in the ankle when standing than when measured in a supine position.[338] In addition, bias can be introduced in any fluid-filled system due to the compliance or length of tubing.[113] As the bias was repeatable, it may be an anatomic variation that can be adjusted for with a correction coefficient.

Although femoral pressures were shown to correlate well with IAP measurements, the femoral blood flow velocity and venous diameter were not significantly altered throughout the experiment. It would be expected that changes would be observed in femoral flow dynamics based on the Hagen-Poiseuille equation that governs flow of an incompressible fluid. Femoral flow and diameter have been determined in both standing horses and horses under general anesthesia, and have demonstrated alterations in flow and diameter with changing hemodynamics.[339,340]

However, these investigators noted the large size of the vessel prevents the ultrasound's Doppler sample gate length from encompassing the entire vessel, and uniform insonation cannot be performed in a horse with the technology available today. The exclusion of the low velocity flow near the wall of the femoral vein may also overestimate the mean velocity and volumetric flow. While the true velocity cannot be determined, changes in blood flow are still detectible.[340,341] As measurements of diameter of the femoral vein are not affected by IAP, the error in flow dynamic calculations is likely to be found in the inaccuracies of the flow measurements.

Measurement of IAP in humans is commonly performed using as intra-vesicular catheter to obtain indirect measures of pressure as they are transmitted through the wall of the bladder.[113] In horses, indirect measurement of IAP using bladder and gastric measurements of pressure have fallen short due to the high variance and lack of repeatability within and between horses.[11-14] Currently, only direct IAP measurement is accepted in horses using a peritoneal cannula, needle, or a microsensor catheter placed in the peritoneal cavity.[11,16] However, the invasiveness of the procedure, as well as the risk of infection or injury to the abdominal organs, has limited its use in the clinical patient. Intra-abdominal hypertension has been described in horses, and a simpler diagnostic tool is needed to allow for identification and management of this syndrome.[10] As femoral pressures show a good correlation with IAP, catheterization of the femoral vein may provide a less invasive method to monitor IAP in this species.

The minimal changes noted in heart rate, MAP, and respiratory rate were expected, and the respiratory rate reflected the moderate elevations in intra-abdominal pressure obtained by insufflation transmitted across the diaphragm.[80,298,336] Horses have

previously shown reductions in heart rate when insufflated to 15 mmHg with carbon dioxide, however, these horses were also administered a bolus of detomidine (20 µg/kg), which caused bradycardia shortly after administration.[328,342] A second report where horses were also insufflated to 15 mmHg that used a constant rate infusion of detomidine (8.54 µg/kg/hr) did not show significant changes in heart rate over time, although baseline heart rate without sedation was not obtained.[223] A preliminary study performed by our laboratory previously examined the effects of a bolus of 10 µg/kg detomidine hydrochloride and a bolus of detomidine (10 µg/kg) with butorphanol (10 µg/kg) on IAP and cardiorespiratory parameters up to 90 minutes post-sedation in normal, healthy horses.[343] In this report, horses administered the drug did not have any significant change in heart rate compared to baseline or the saline controls. (Figure 6-14) These horses were not insufflated. The current investigation, which used a constant rate infusion of detomidine, also noted no significant change in heart rate, consistent with our previous investigation, and that by Cruz et. al.[223]

MAP was not significantly altered in horses during the experiment from baseline levels measured prior to insufflation. Previous studies, including the preliminary study performed in our laboratory, have noted a significant decrease in MAP after administration of a detomidine bolus of 10 µg/kg or higher.[328,343] (Figure 6-15) However, pharmacologic reports evaluating alpha 2 agonists in horses have noted no difference in MAP from baseline levels with detomidine doses of 10 µg/kg.[344] Transient increases in MAP were only noted at higher doses of the drug in that study, and in other reports.[345] A study evaluating the effects of abdominal insufflation on cardiorespiratory parameters in normal, standing horses demonstrated that a constant rate infusion of detomidine (8.54

µg/kg/hr) did not alter MAP over the course of the experiment, with or without insufflation to 15 mmHg.[223] Therefore, based on the extensive publications on the pharmacologic effects of detomidine demonstrated in other studies, it is unlikely that the doses of this drug used in this study in a constant rate infusion had any effect on MAP in the current investigation. In regards to the effects of IAP on MAP measurements, investigations in other species have shown minimal difference in MAP until abdominal pressures rise above 25 mmHg.[80] Our results are consistent with these findings.

Alpha-2 agonists are mild respiratory depressants. After administration, respiratory rate decreases and tidal volume increases, resulting in minimal changes in arterial pH oxygen and carbon dioxide levels.[346-348] Our preliminary work evaluating the effects of detomidine in horses was consistent with these reports.[343] (Figure 6-16) Conversely, the horses in the current study became tachypneic, and the obvious abdominal distention appeared to be the cause as increases in IAP will shift the pressure volume curve to the right.[336] Both functional residual capacity and static compliance of the chest are reduced by IAH, and abdominal pressures as low as 15 mmHg have been reported to produce hypoxemia and hypercapnia.[88-90] While blood gas analysis was not performed in the current study, adverse changes were not seen in healthy horses insufflated up to 15 mmHg, although a hyperoxia was been reported.[223,328]

Central venous pressure is cited as an indicator of the interaction between the cardiac pump and the vascular circuit returning the blood to the heart.[241-243] It is the mean vena cava or right atrial pressure, equivalent to right ventricular end diastolic pressure in the absence of tricuspid stenosis. As right atrial pressure just prior to the onset of systole determines the preload of the right ventricle as well as cardiac output, CVP measurement

has been utilized as a determinant of cardiac function and venous return to the heart.[249] CVP has been recommended as an endpoint in early goal directed therapies in humans, along with mean arterial pressures and central venous oxygen saturation, to significantly decrease mortality rates in patients admitted with septic shock.[64]

Recent metanalytic reviews have noted that early goal directed therapy in patients with septic shock provided no benefit in regards to outcomes and survival.[65,349] One review noted that goal directed therapy actually increased the mortality rate, which may have been associated with an increased volume of fluids administered and an increase in the number of red cell transfusions.[350] It is suspected that decreased cardiac function, primarily left ventricular dysfunction, and subsequently right ventricular overload, common in patient with septic shock, plays a role in altering CVP.[287] Even in healthy patients, CVP cannot predict the response to volume loading, as it cannot accurately reflect ventricular filling volume or cardiac function.[288]

Elevated IAP is noted to increase intra-thoracic pressures, and falsely elevate CVP well as pulmonary artery occlusion pressures. [47,66,76,81,83,292] In these cases with IAH, continued fluid resuscitation often resulted in improved cardiac output and organ function, despite the intuitive contraindications based on only observed measures of intra-vascular volume. Conversely, failure to administer fluids can result in under-resuscitation, and hasten the onset of multiorgan failure.[81] Measurements of CVP and pulmonary artery occlusion pressures are artificial in the face of IAH, and are not an accurate representation of the patient's intravascular volume status.[66,81,83]

In humans, volumetric monitoring parameters are considered more useful in identifying cardiac filling and output in the face of IAH and increased intra-thoracic

pressure.[74,81,351] These can include right ventricular end diastolic volume index or global

end diastolic volume index.[81,352] Response to fluid bolus is best determined using pulse

pressure variation or stroke volume variation.[351] However these monitoring parameters

require a sedated and ventilated patient, and arrhythmias negate the ability to interpret the

results.[353,354] Because of the difficulty in obtaining these measurements in our veterinary

species, it is recommended to estimate CVP by adjusting for the thoracic pressure. The

intra-thoracic pressure can be estimated as 50% of the intra-abdominal pressure measured

from the abdominal cavity.[355] In clinical cases of horses with an elevated CVP, it would

be recommended to assess IAP to determine if adjustments should be made in this raw

measurement to guide resuscitation efforts. IAP measurements may also help to predict if

the CVP could be affected by a zone 2 abdomen.

In summary, this study evaluated the effects of increasing intra-abdominal

pressure in healthy horses on hemodynamic variable including CVP, FVP, femoral blood

flow and diameter. Results of our study are clinically relevant in that they further define

the limitations and interpretation of CVP values. Correlations between FVP and IAP

may also improve the quality of patient care, by providing a less invasive method for

monitoring for IAH. Further work is needed to better define the level of IAP that

significantly inhibits venous return, and causes a zone 2 abdomen. Many commonly

identified disorders, including peritonitis, large colon displacement and volvulus, hydrops

and ascites a may alter abdominal pressure in the horse. Therefore, elevations in CVP

should be interpreted carefully with deference to the disease process being monitored and

alterations in IAP measurements.

Table 6-1 Signalment of horses selected.

Horse	Breed	Age (years)	Weight (kilograms)	Body Condition Score
1	American Warmblood	15	596.3	7
2	American Quarter Horse	19	496.4	6.5
3	American Quarter Horse	11	584.5	5
4	American Quarter Horse	25	445	5
5	American Quarter Horse	17	567.3	6
6	Tennessee Walking Horse	8	563.6	7.5
7	Thoroughbred	15	531.8	7

Figure 6-1 Boxplot (median, 25th and 75th quartiles, range) of comparisons of heart rate in beats per minute over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Heart rate was not significantly different for any timepoint (P=0.3985).

Comparison of Heart Rate over Time with Increasing Intra-Abdominal Pressure

Figure 6-2 Boxplot (median, 25th and 75th quartiles, range) of comparisons of respiratory rate in breaths per minute over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Respiratory rate was not significantly different for any timepoint (P>0.1197).

Comparison of Respiratory Rate over Time with Increasing Intra-Abdominal Pressure
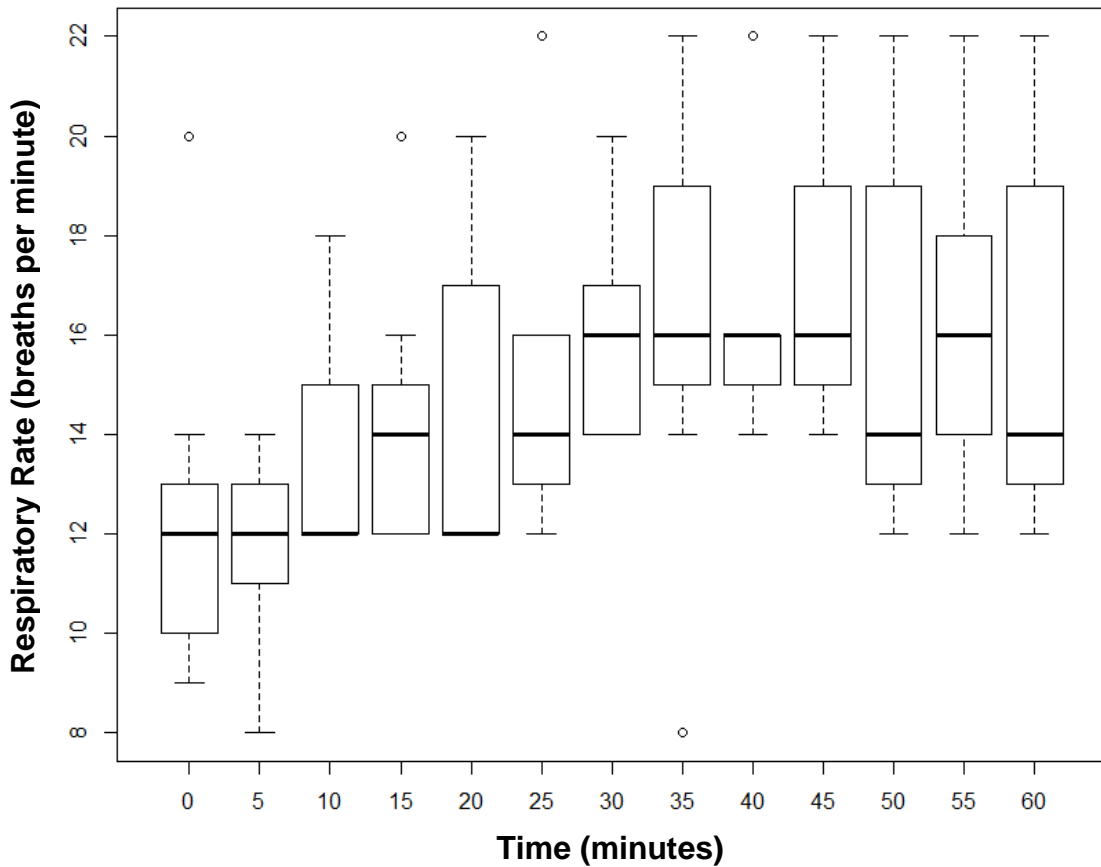
Figure 6-3 Boxplot (median, 25th and 75th quartiles, range) of comparisons of mean arterial blood pressure in mmHg over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Mean arterial blood pressure was not significantly different for any timepoint (P>0.13).

Comparison of Mean Arterial Blood Pressure over Time with Increasing Intra-Abdominal Pressure
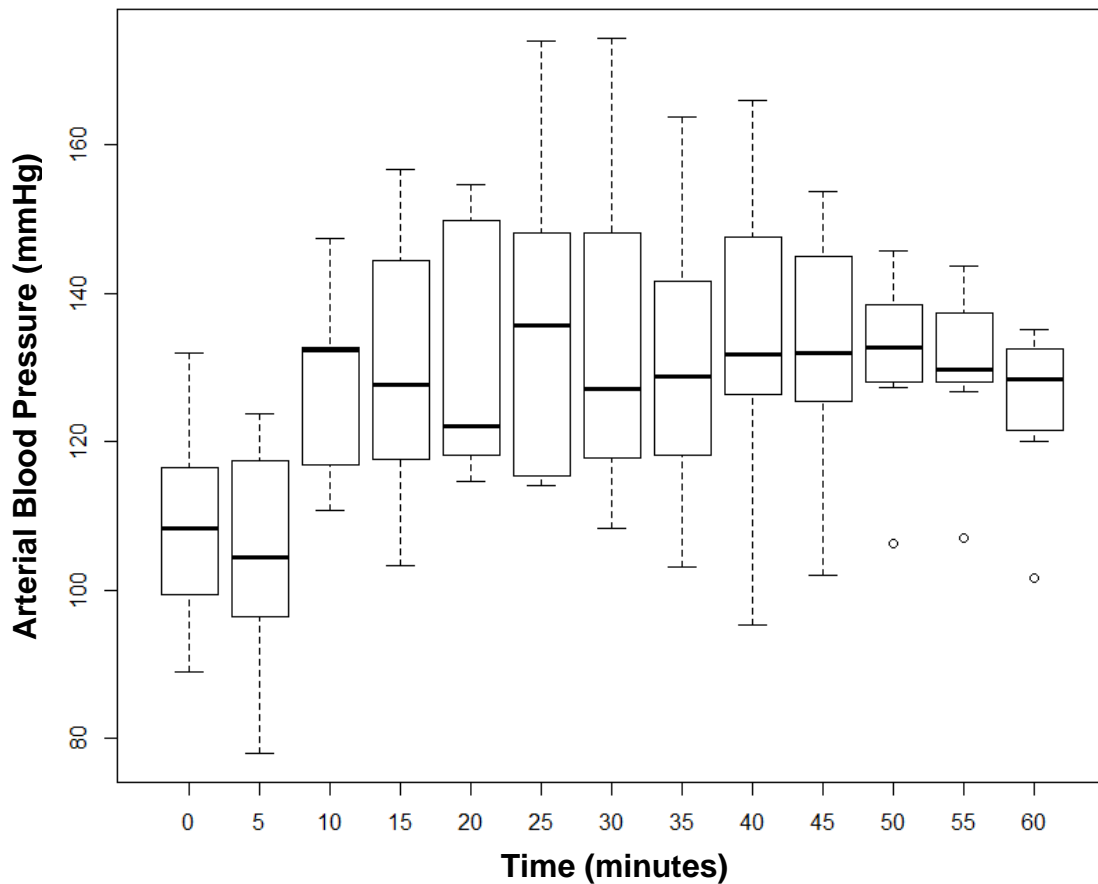
Figure 6-4 Boxplot (median, 25th and 75th quartiles, range) of comparisons of systolic central venous pressures over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Systolic central venous pressures were not significantly different for any timepoint (P=0.06846).

Comparison of Systolic Central Venous Pressure over Time with Increasing Intra-Abdominal Pressure
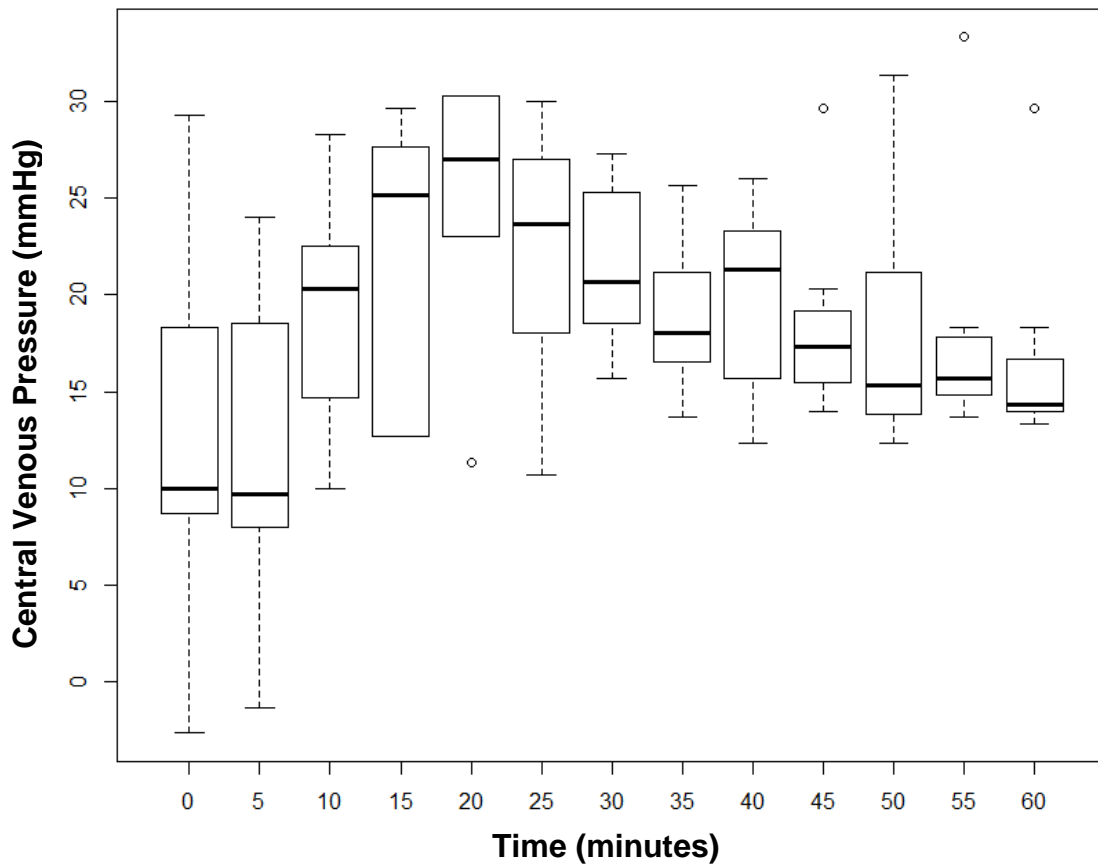
Figure 6-5 Boxplot (median, 25th and 75th quartiles, range) of comparisons of mean central venous pressures over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Mean central venous pressures were not significantly different for any timepoint (P=0.2618).

Comparison of Mean Central Venous Pressure over Time with Increasing Intra-Abdominal Pressure
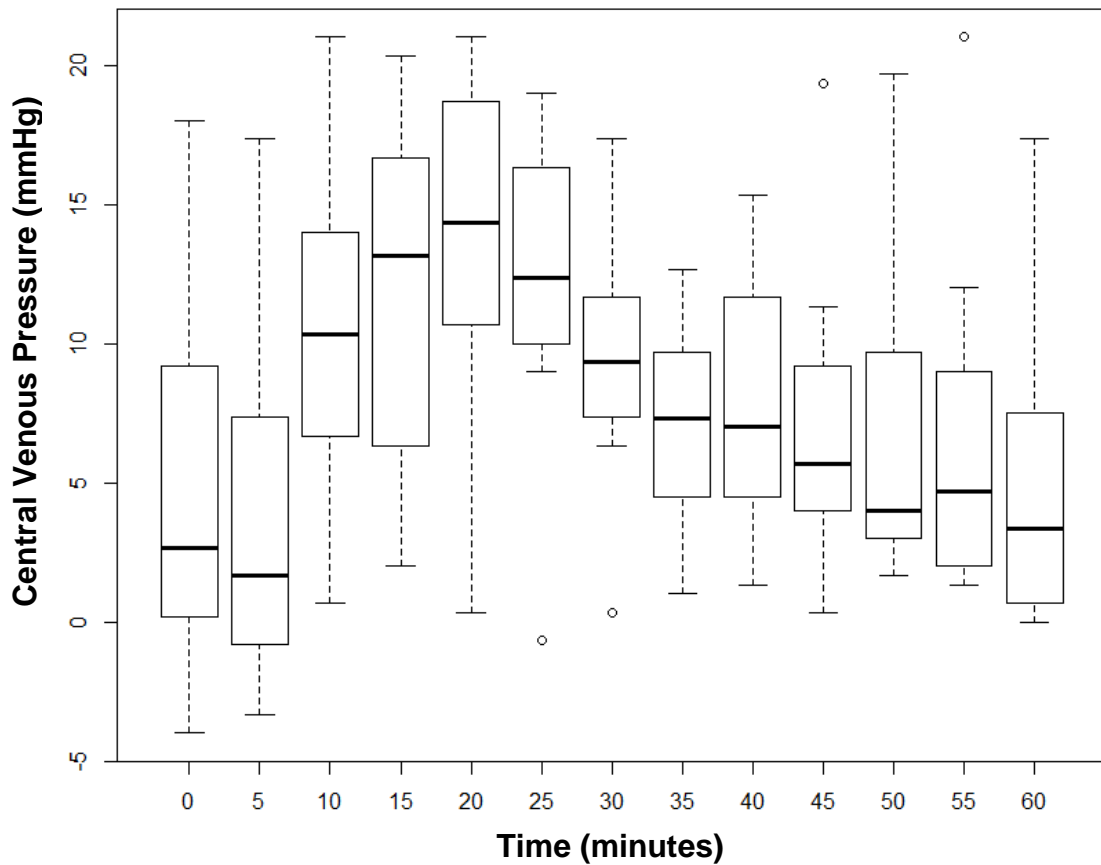
Figure 6-6 Boxplot (median, 25th and 75th quartiles, range) of comparisons of diastolic central venous pressures over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Diastolic central venous pressures were not significantly different for any timepoint (P=0.3528).

Comparison of Diastolic Central Venous Pressure over Time with Increasing Intra-Abdominal Pressure
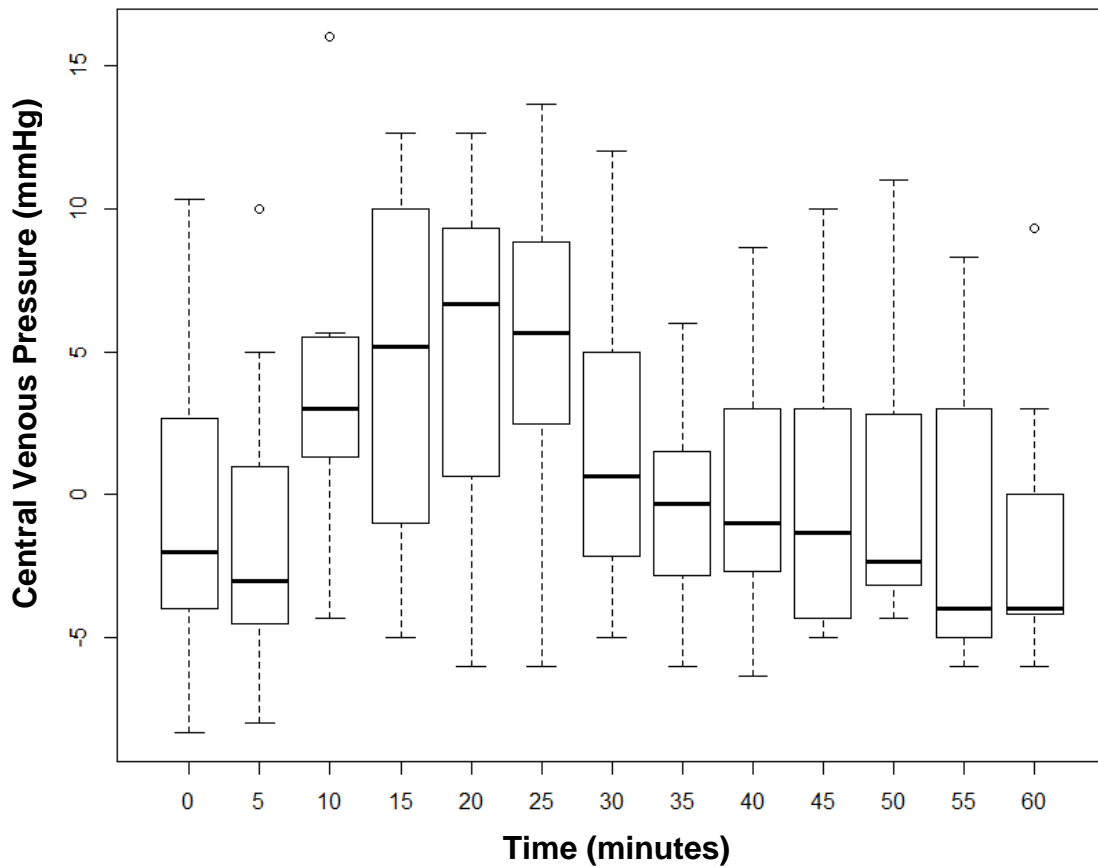
Figure 6-7 Boxplot (median, 25th and 75th quartiles, range) of comparisons of femoral vein diameter in centimeters (cm) over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Vein diameter was not significantly different for any timepoint (P=0.9963).

Comparison of Femoral Vein Diameter over Time with Increasing Intra-Abdominal Pressure
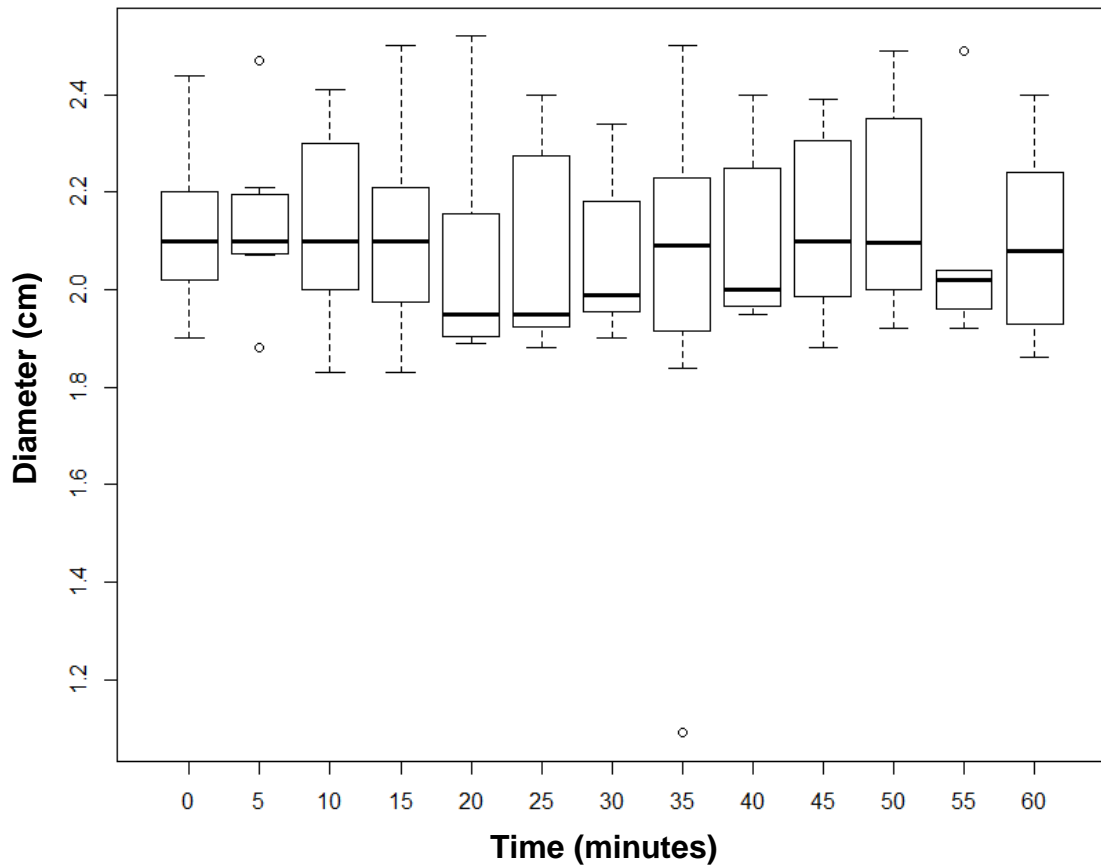
Figure 6-8 Boxplot (median, 25th and 75th quartiles, range) of comparisons of peak average velocity in the femoral vein in centimeters per second over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Velocity was not significantly different for any timepoint (P=0.9993).

Comparison of Peak Average Velocity over Time with Increasing Intra-Abdominal Pressure
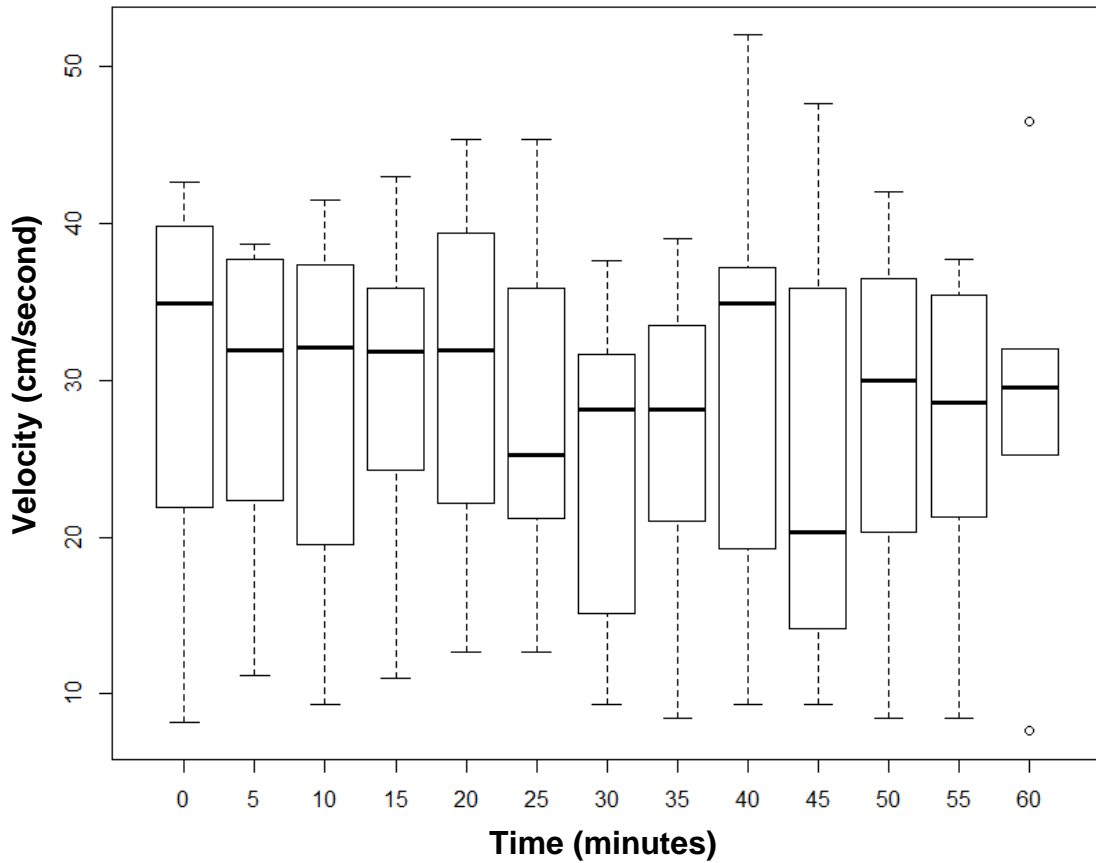
Figure 6-9 Boxplot (median, 25th and 75th quartiles, range) of comparisons of time averaged velocity in the femoral vein in centimeters per second over time. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Velocity was not significantly different for any timepoint (P=0.9306).

Comparison of Time Averaged Velocity over Time with Increasing Intra-Abdominal Pressure
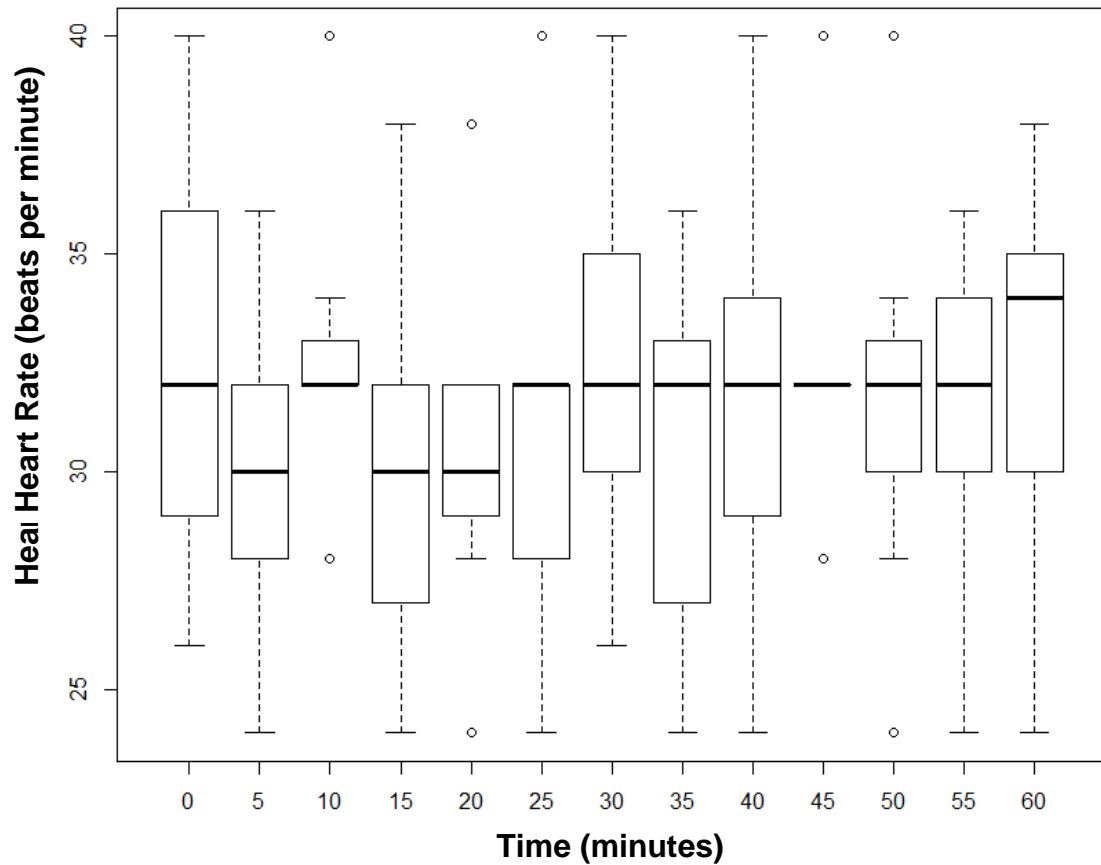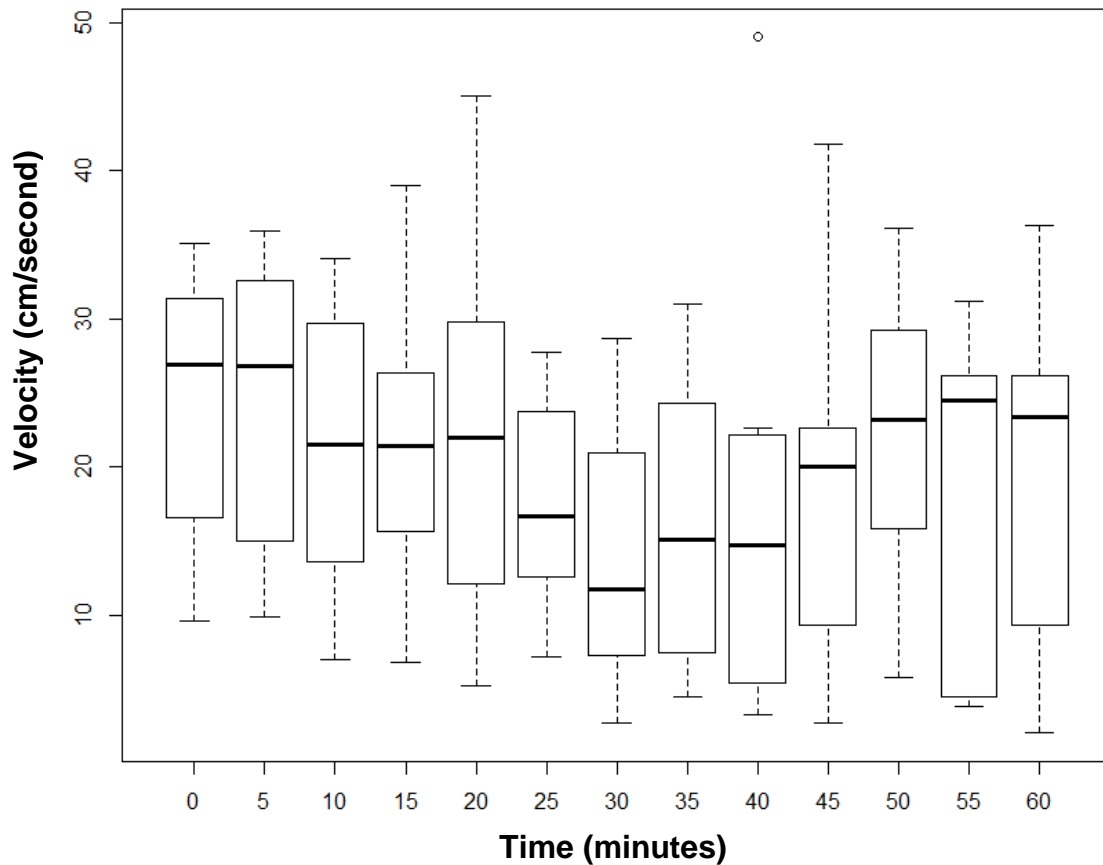
Figure 6-10 Boxplot (median, 25th and 75th quartiles, range) of comparisons of venous pressure in mmHg over time. Both venous pressure and pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg. Timepoints with different letters are significantly different (P<0.00341).

Comparison of Femoral Venous Pressure over Time with Increasing Intra-Abdominal Pressure
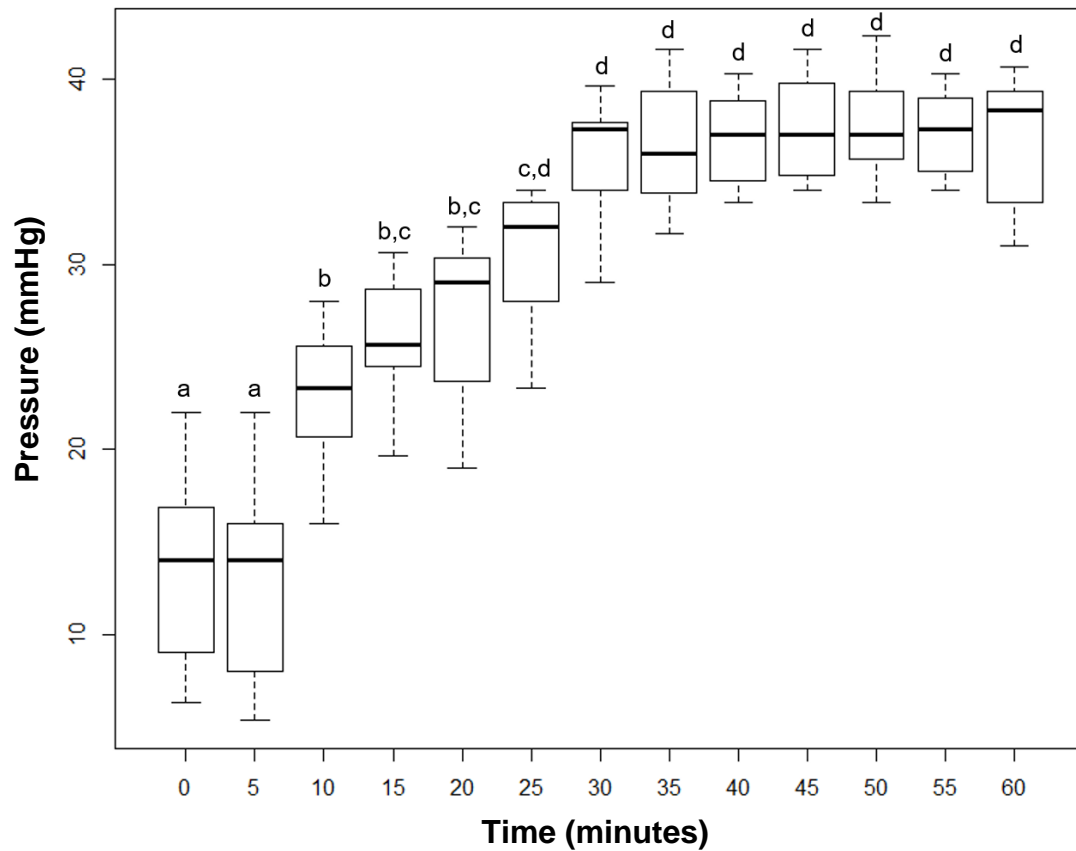
Figure 6-11 Bland Altman plot of the differences between techniques in mmHg against the average of the two techniques as a comparison of the pressures measured by the insufflator versus direct abdominal pressure measurement. Direct measurement overestimated the insufflation pressure (bias 1.9 mmHg, LOA: -2.3 mmHg, 6.1 mmHg).
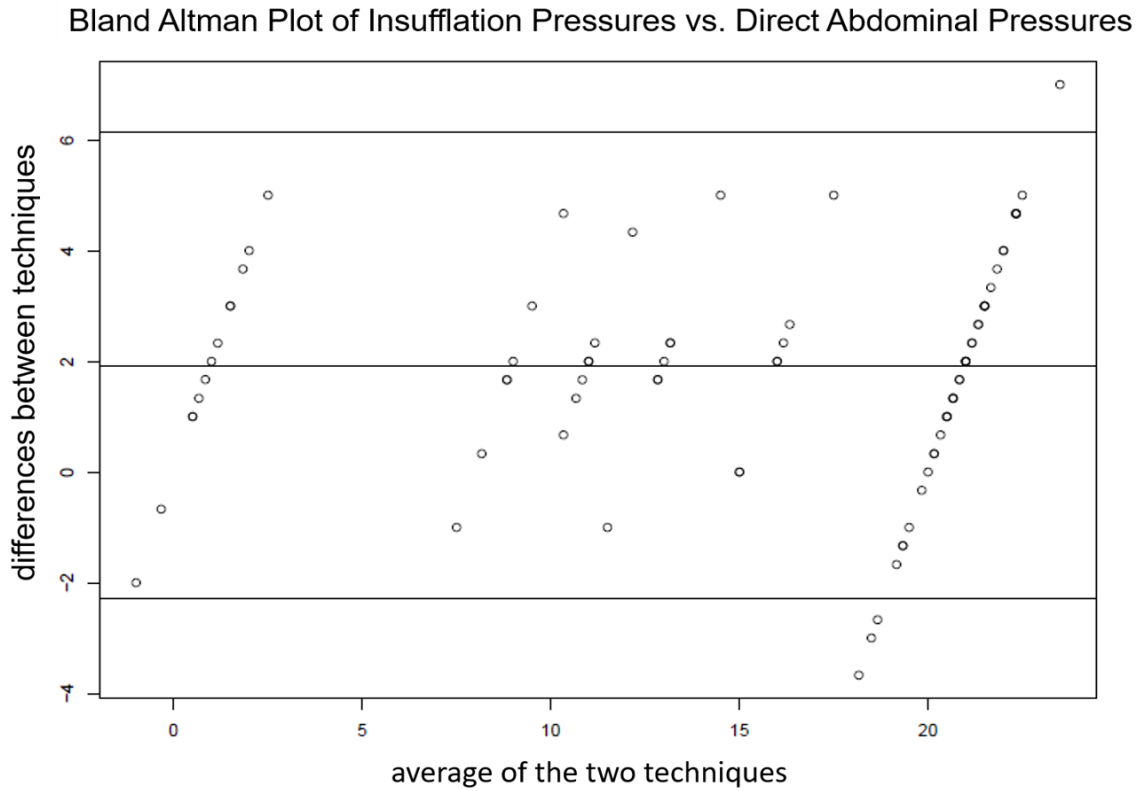
Figure 6-12 Bland Altman plot of the differences between techniques in mmHg against the average of the two techniques as a comparison of the pressures measured by the femoral venous catheter versus direct abdominal pressure measurement. Direct measurement overestimated the insufflation pressure (bias 13.8 mmHg, LOA: 6.5 mmHg, 21.1 mmHg).

Bland Altman Plot of Femoral Venous Pressures vs. Direct Abdominal Pressures
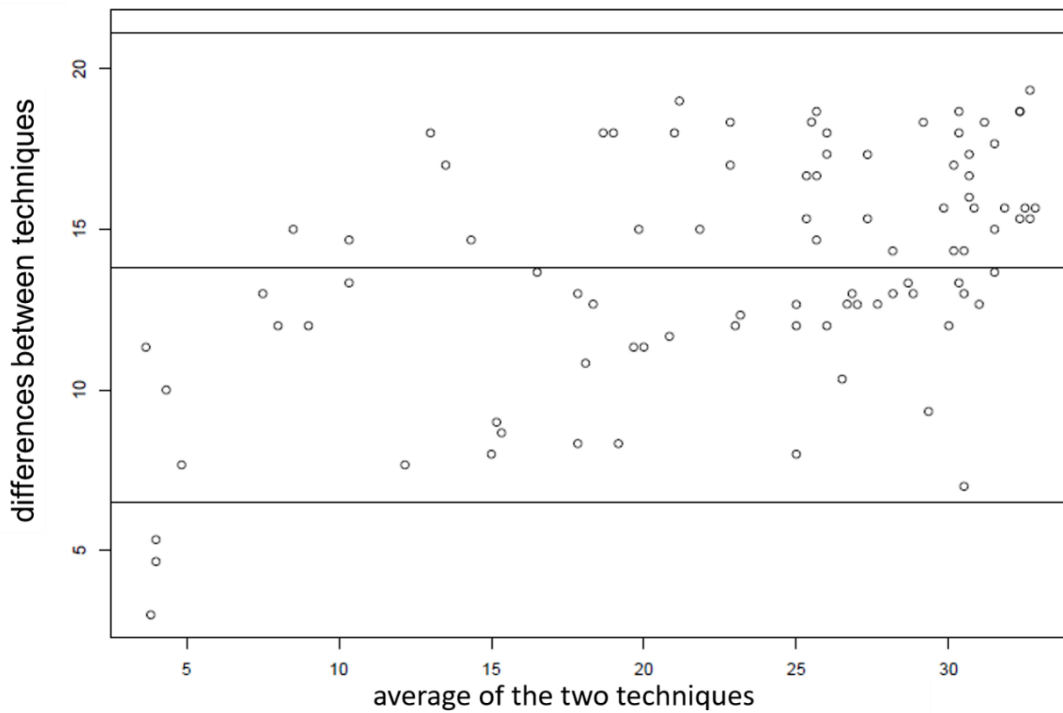
Figure 6-13 Bland Altman plot of the differences between techniques in mmHg against the average of the two techniques as a comparison of the pressures measured by the femoral venous catheter versus pressures measured by the insufflator. Direct measurement overestimated the insufflation pressure (bias 15.7 mmHg, LOA: 7.4 mmHg, 24.1 mmHg).

Bland Altman Plot of Insufflation Pressures vs. Femoral Venous Pressures
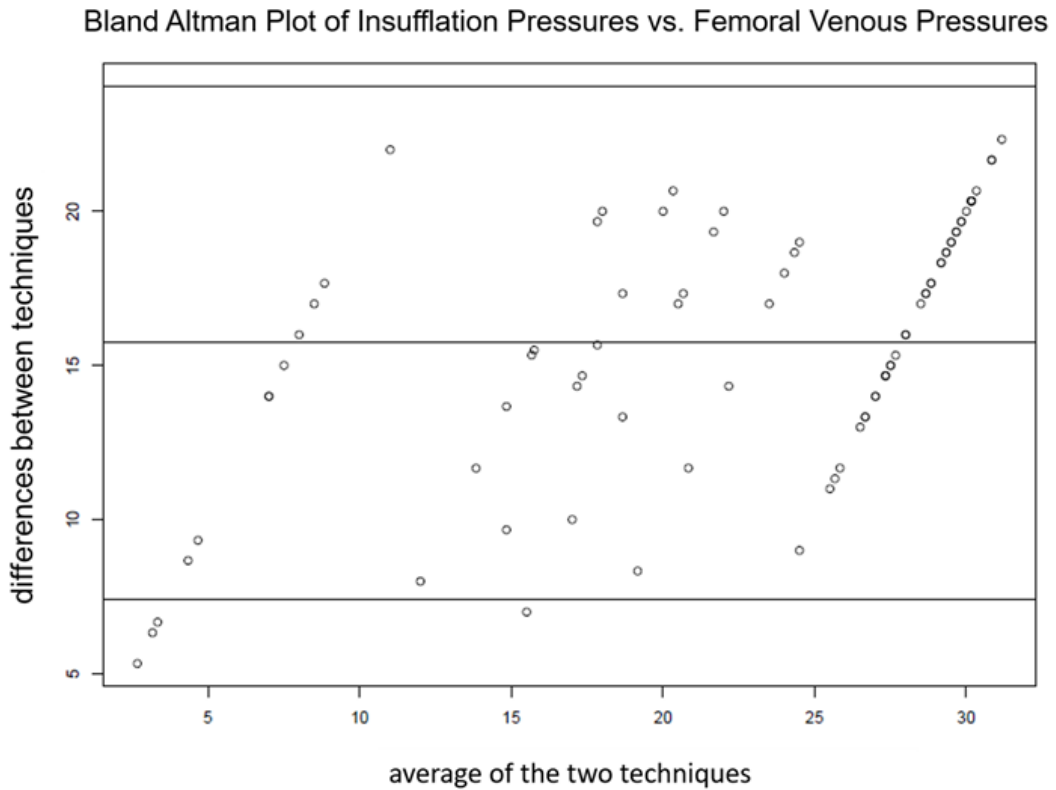
Figure 6-14 Boxplot (median, 25th and 75th quartiles, range) of comparisons of heart rate over the 90-minute observation period for the saline control (C), detomidine (D) and detomidine and butorphanol (DB) treatments in the preliminary study. Treatment with saline or detomidine did not significantly change heart rate compared to baseline (T0). Heart rate was significantly decreased after administration of detomidine and butorphanol (DB) from minute 1 to 20 (T1-T2) (P<0.05). Each time period on the x axis represents a 10-minute increment except time 0, which represents 5 minutes. For each box, the horizontal line represents the median, and the upper and lower boundaries of the box represent the 75th and 25th percentile, respectively. The whiskers represent the upper and lower quartiles +/- 1.5 times the interquartile range. Asterisks represent outliers.

Heart Rate over Time in Normal Horses
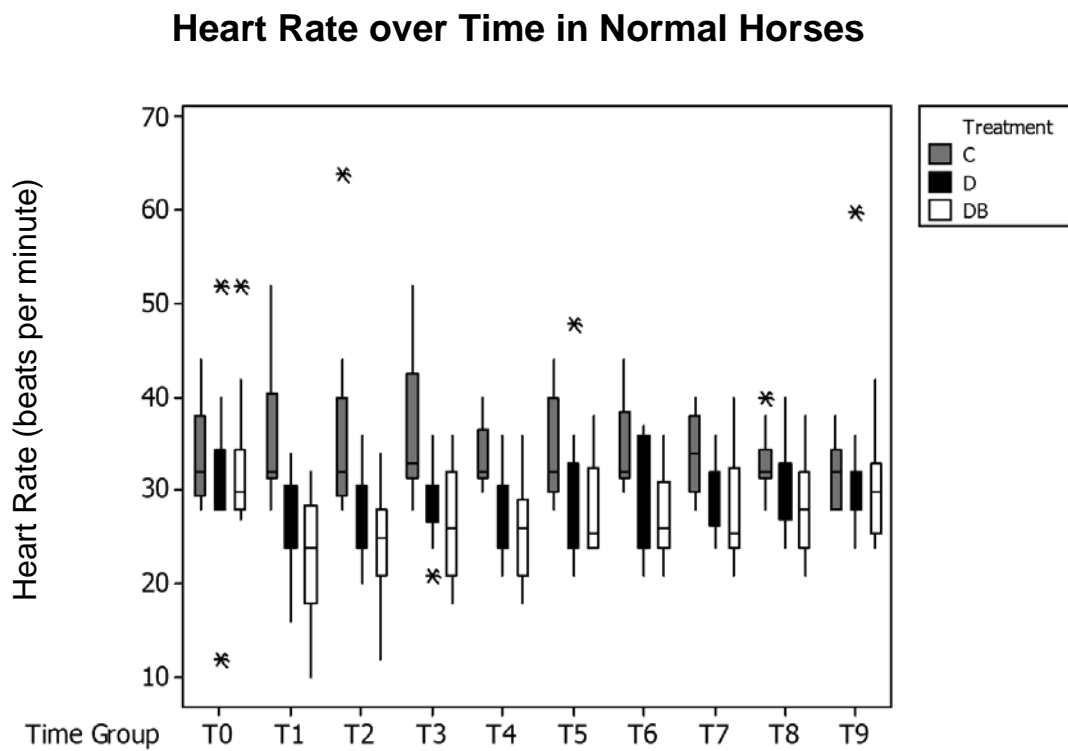
Figure 6-15 Boxplot (median, 25th and 75th quartiles, range) of comparisons of mean arterial pressure (MAP) in mmHg over the 90-minute observation period for the saline control (C), detomidine (D) and detomidine and butorphanol (DB) treatments in the preliminary study. Detomidine (D) significantly decreased MAP from 41 to 90 minutes after drug administration (T5 to T9) (P<0.05). The other medications had no effect. Each time-period on the x axis represents a 10-minute increment except time 0, which represents 5 minutes. For each box, the horizontal line represents the median, and the upper and lower boundaries of the box represent the 75th and 25th percentile, respectively. The whiskers represent the upper and lower quartiles +/- 1.5 times the interquartile range. Asterisks represent outliers.

Mean Arterial Pressure over Time in Normal Horses
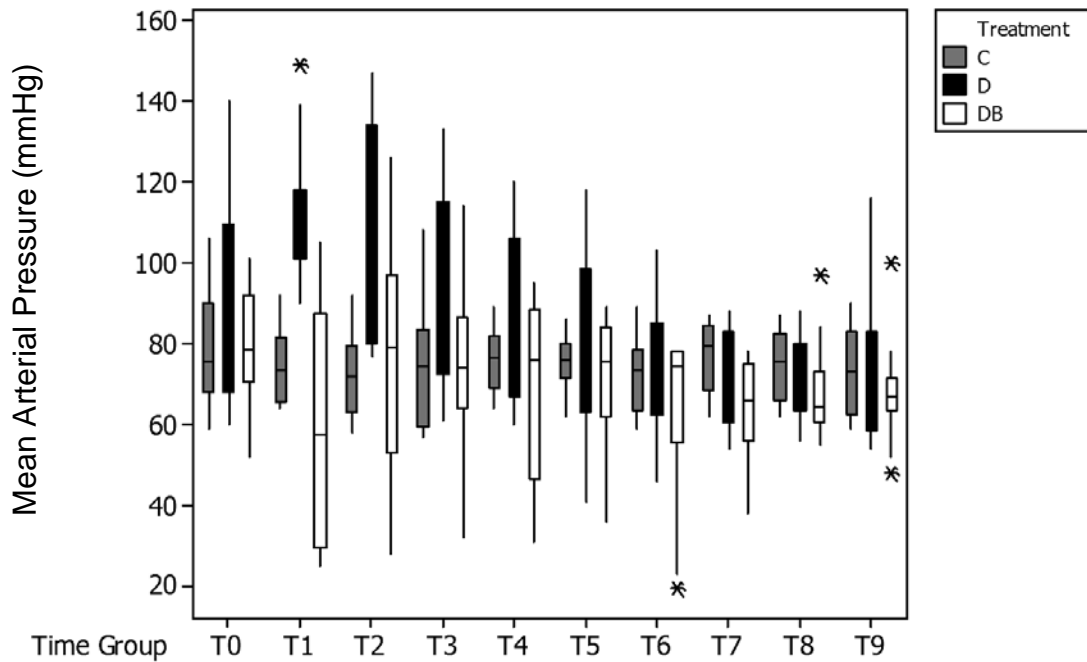
Figure 3-16 Boxplot (median, 25th and 75th quartiles, range) of comparisons of respiratory rate (RR) in breaths per minute over the 90-minute observation period for the saline control (C), detomidine (D) and detomidine and butorphanol (DB) treatments in the preliminary study. Detomidine (D) significantly decreased respirations for all time periods compred to baseline, whereas detomidine and butorphanol (DB) decreased respirations from minute 21 to minute 90 (T3-T9) ($P<0.05$). Each time-period on the x axis represents a 10-minute increment except time 0, which represents 5 minutes. For each box, the horizontal line represents the median, and the upper and lower boundaries of the box represent the 75th and 25th percentile, respectively. The whiskers represent the upper and lower quartiles +/- 1.5 times the interquartile range. Asterisks represent outliers.

Respiratory Rate over Time in Normal Horses
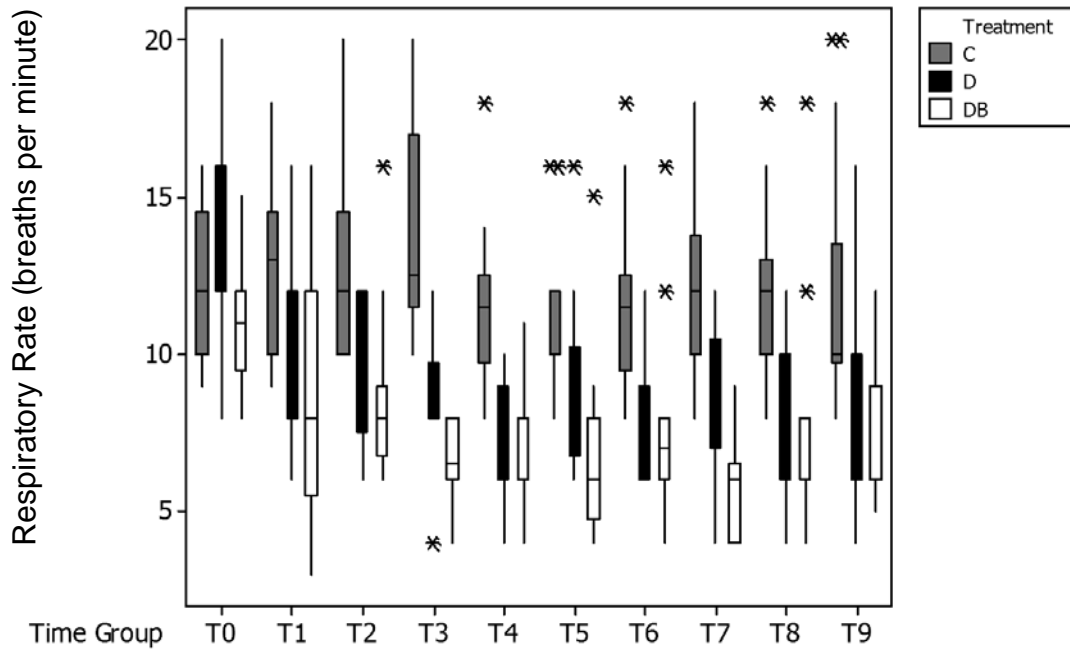
Chapter 7

7.1   Introduction

Biomarkers are defined as an objective measure of normal or pathologic biological processes or pharmacologic responses to therapeutic interventions that can be obtained from outside the patient.[356,357] It is a requirement that these markers have the ability to be measured accurately and reproducibly. There are several classifications of biomarkers defined by the FDA-NIH Biomarker Working Group.[358] Diagnostic biomarkers are used to identify or confirm the presence of a disease or condition. Monitoring biomarkers are measured serially to track a disease or to monitor for the effect of an intervention. Predictive biomarkers identify an individual's likelihood (or lack thereof) of a response to an effect or treatment. Prognostic biomarkers identify the likelihood of an event recurrence or progression of a condition. Finally, susceptibility biomarkers predict the potential of a medical condition to occur. These classifications can overlap, and a biomarker can fall into more than one group or category.

Serum markers have been used extensively in horses for evaluation of the presence of progression of musculoskeletal disorders and metabolism, neoplasia, inflammatory conditions, reproduction and cardiac disease.[359-362] For example, troponin I is a sensitive and specific biomarker of myocardial insults and arrhythmias, which can be

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used for diagnosis and monitoring of injury due to myocardial disease, trauma, and the toxic effects of envenomation, among others.[363-365] It has also been suggested to serve as a prognostic indicator for acute gastrointestinal illness.[366] However, the lack of specificity of some markers can hinder differentiation between disease processes or their source. One such biomarker is serum amyloid A, an acute phase protein released into the bloodstream and peritoneal fluid in inflammatory conditions. Serum levels of serum amyloid A are known to rise in the face of gastrointestinal diseases, such as colic, alongside other acute phase proteins including haptoglobin.[367,368] However, it can also increase with localized infections, such as septic arthritis, suggesting it is not specific to gastrointestinal disease.[369]

7.2   Biomarkers of Intra-Abdominal Hypertension

Intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS) are life-threatening conditions that can increase mortality in critically ill patients.[1,19] As intestinal dysfunction is one of the initial signs of ACS due to hypoperfusion, early identification of intestinal ischemia could hasten diagnosis and provide guidance for therapeutic intervention.[100-103] While an elevated IAP is necessary for identifying the disease, increased levels by themselves do not consistently identify the onset of ACS.[7,47,48] A biomarker of acute intestinal ischemia would improve the identification of this disease. There are few reports currently in the literature investigating biomarkers for IAH and ACS.

7.2.1   D-Lactate

D-lactate is produced by bacterial metabolism, and cannot be produced by mammals. It is released from the gastrointestinal lumen due to increased intestinal mucosal and capillary permeability, and as the liver does not metabolize it, D-lactate is more specific for identification of anaerobic metabolism than its stereoisomer L-lactate.[370,371] In a report by Nielsen et. al., D-lactate was shown to increase in the peripheral bloodstream within 240 minutes of initiation of IAH using a porcine pneumoperitoneum model.[372] These findings correlating increased circulating D-lactate with IAH were also reported in an IAH model in rats.[373] In horses, plasma D-lactate has only been investigated in gastrointestinal disease, and was unable to differentiate between strangulating and non-strangulating obstruction.[374] However, D-lactate showed good sensitivity and moderate specificity for identification of strangulations.[374] This was contradicted by a separate study, where plasma D-lactate concentrations were not different between a population of horses with and without colic, indicating the effect of IAH on biomarkers may have species and sampling differences.[375]

7.2.2   N Terminal Fragment of Brain-Type Natriuretic Peptide

Cardiovascular biomarkers of IAH have also been investigated in a porcine pneumoperitoneum model. Elevations in the serum levels of N terminal fragment of brain-type natriuretic peptide were observed, corresponding to an increase in systemic and pulmonary vascular resistance.[376] As the N terminal fragment of brain-type natriuretic peptide is released in response to ventricular stretch or pressure overload, the authors suspected the cause was ventricular compromise secondary to the

pneumoperitoneum. To the authors knowledge this biomarker has not been investigated in the horse, as a test for the equine protein does not exist.[377]

7.2.3   Adenosine

A recent study in human surgical patients diagnosed with IAH noted a direct, positive correlation between serum levels of the anti-inflammatory cytokine interleukin-10 (IL-10) and the nucleoside adenosine with IAP above 15 mmHg.[378] Adenosine is released from the vascular endothelium in response to microcirculatory injury and secondary to tissue hypoxia. All adenosine receptors are G protein cell surface receptors, coupled to a G_s alpha subunit that stimulates adenylate cyclase and production of cAMP.[379-382]   Although at least 4 subtypes of adenosine receptors have been identified (A_1, A_{2A}, A_{2B}, and A_3), the primary receptor involved in modulation of inflammation is A_{2A}.[382-384]

Stimulation of the A_{2A} receptor by adenosine has been shown in horses and other species to decrease adherence of neutrophils, and attenuate both increases in capillary permeability and production of superoxide radicals caused by neutrophil binding.[384-386] Induction of inflammatory mediators, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-1β (IL-1β) can be reduced by activation of the A_{2A} adenosine receptor on a variety of cells, including macrophages, Kupffer cells, and monocytes.[384,385] Interaction with adenosine receptors on T lymphocytes can induce T cell anergy and adaptive T cell responses, despite the presence of co-stimulatory molecules such as IL-1.[387] Release of adenosine may also provide modulation of peripheral tolerance, preventing reaction to self-antigens released by tissue

injury.[388,389] In horses, both lipopolysaccharide and TNF-α have been noted to modulate the expression of adenosine receptors on the surface of peripheral blood monocytes, increasing the density of A_{2A} receptors and the affinity of a cell to respond to adenosine agonists.[383]

While adenosine receptors have been shown to modulate both inflammation and the adaptive immune response, they have also been noted to play a role in hepatosplanchnic blood flow during sepsis, promoting vasodilation in the face of ischemia.[390] There is some thought that elevated adenosine concentrations may exacerbate IAH, by pooling of blood in the splanchnic vessels and tissues by a reduction in vascular resistance.[378,391] In contrast, renal vasculature dilatation has been identified to be modulated by the A_{2A} adenosine receptor globally, but the A_1 receptor locally, resulting in contraction of the afferent renal arterial in response to reductions in renal perfusion.[392] Together, these normally provide for paracrine control of glomerular function and homeostasis, but may fail in the face of IAH, where compression of the renal vasculature and increased renal vascular resistance overwhelm the glomerular-tubular feedback mechanism.[393-395]

Recent investigations into the modulation of the adenosine in the face of IAH have been performed in critically ill human patients, using the intravenous theophylline, an adenosine A_1 and A_{2A} receptor antagonist.[391] In clinical patients with IAP at or above 12 mmHg, twice daily infusions of theophylline (200 mg IV, BID for 5 days; serum levels: 10 to 20 μg/ml) dramatically improved survival from 45% in the untreated patients to 100% survival in the treated group. Both serum IL-10 and adenosine, identified as biomarkers of IAH from a previous publication,[378] were reduced by

treatment with theophylline. By day 5, the IAP was no longer hypertensive, suggesting a resolution of IAH secondary to medical management.

7.2.4   Interleukin-10

Interleukin-10 (IL-10) is an inhibitory cytokine produced by macrophages secondary to stimuli including circulating endotoxin, TNF-α, and catecholamines.[396] IL-10 modulates the inflammatory response by reducing the production of pro-inflammatory cytokines, chemokines, and cell adhesion molecules including IL-1 alpha and beta, IL-6, IL-8, TNF-α, and granulocyte colony stimulating factor (GCSF) at the level of transcription.[397-401] It also induces the production of anti-inflammatory mediators, including IL-1 receptor antagonist. In addition, the synthesis of cyclooxygenase-2 and the production of prostaglandin E_2 is inhibited by IL-10.[402] IL-10 stimulates the proliferation of B lymphocytes to counter bacterial toxins and provides improved local mucosal defense.[397] In humans with sepsis, IL-10 serves as a marker for overwhelming infection, as increased serum levels are associated with a poor prognosis, increased risk of multi-organ failure, and death.[403-405] IL-10 is associated with immunodepression in trauma, burn and surgery patients, predisposing them to infectious complications if overexpressed.[406,407]

Monocytes from adult horses with gastrointestinal disease were found to produce IL-10 when exposed to lipopolysaccharide (LPS), and plasma levels increase rapidly after systemic infusion of LPS in the horse.[408,409] Increased production of mRNA for IL-10 in blood leukocytes is rapid; it begins in the first hour after exposure to LPS, and spikes again at 5 to 6 hours after exposure.[410] The production of IL-10 was higher in monocytes

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from horses with gastrointestinal disease that died and in horses with strangulating lesions, suggesting that a trend towards a pro-inflammatory phenotype (and therefore lower levels of IL-10) is associated with survival.[409,411] The cause is suspected to be due to activation of adenosine A_{2A} receptors on the monocyte, modulating the action of LPS induction of IL-10 production. Incubation of equine LPS stimulated peritoneal macrophages with human IL-10 resulted in a dose dependent reduction in the secretion of TNF-α, IL-6, and prostaglandin E_2.[412] TNF-α was found to be the most sensitive cytokine to the effects of recombinant IL-10 in the horse. Similar findings were noted in mice, where IL-10 prevented mortality with an associated reduction in TNF-α and interferon gamma, when challenged with LPS dosed at the LD50.[413,414] When LPS is administered systemically to horses, circulating IL-10 levels were found to peak at one hour after administration, slowly declining to levels similar to baseline at 2 hours.[415]

In foals with sepsis, serum IL-10 was not noted to be elevated compared to healthy foals, but the highest level measured was associated with mortality in one foal.[416] However, both non-surviving septic and sick foals were noted to have an increasing gene expression of IL-10 in isolated peripheral blood mononuclear cells, indicating a possible role for IL-10 in non-survival.[417] The production of IL-10 in response to LPS and interferon gamma appears to be associated with age, as neonatal cells demonstrate a two-fold increase in mRNA production compared with adult monocytes, macrophages and dendritic cells.[418]

In a recent study in human surgical patients with increased IAP, IL-10 was the only cytokine measured that was significantly elevated by abdominal hypertension.[378] A significant correlation (r^2: 0.792) was also observed with measurements of IAP when IL-

10 levels were greater than or equal to 15 mmHg (P<0.001). The elevated serum levels of IL-10 were suggested to be secondary to a similar rise noted in serum adenosine, which can stimulate monocyte secretion of IL-10.[419] However, a number of inflammatory mediators can also increase its production.

7.2.5   Intestinal Fatty Acid Binding Protein

Intestinal fatty acid binding protein (IFABP) is a low molecular weight (14-15 kD) cytosolic protein found in tissues involved in the uptake and use of fatty acids.[420,421] IFABP can constitute 2-3% of the cytosolic proteins in intestinal cells, and this protein is most commonly found in the mature enterocytes at the tips of the small intestinal villi.[420] While present throughout the cytosol, it has an affinity for the apical side of the cell in the fasted state.[421] Expression of the IFABP gene has been identified in various tissues of the normal equine gastrointestinal tract, with the jejunum containing the highest concentrations, followed by the ileum and duodenum, demonstrating the presence of this protein in the cells of the mucosa.[420] Expression of mRNA for this protein in the cecum and pelvic flexure as well as the small colon was negligible, and expression was not identified in the stomach mucosa.[420] Based on previous publications, normal concentrations of IFABP are undetectable in the bloodstream of both humans and horses, suggesting that this protein is located intra-cellular without damage of these cells by disease.[420,422]

The small size of the IFABP molecule allows it to leak from enterocytes damaged by trauma or ischemia. However, the stores of IFABP are finite, and they are depleted quickly; in advanced cases of intestinal necrosis, testing may observe little to no

circulating IFABP.[423] In humans, serum, abdominal fluid, and urine concentrations of IFABP have been correlated with early phases of mucosal injury in abdominal disorders including small intestinal volvulus, incarcerated bowel, ischemia, necrotizing enterocolitis, ulcerative colitis, and acute thromboembolic intestinal ischemia.[422-425] In addition, serial measurements were able to accurately predict the development complications in infants with necrotizing enterocolitis.[426]

Serum levels of IFABP were noted to be significantly higher in patients with strangulating small bowel obstruction and ischemia, compared to simple obstructions that did not compromise tissue perfusion.[427-429] However, IFABP is suggested to be a better marker for ischemia secondary to infarction, as venous occlusion may result in sequestration of the marker in the vasculature of strangulated bowel.[424] Alternatively, arterial obstruction that results in ischemia, but does not obstruct venous outflow, may still allow biomarkers an outlet to the circulation, and increased levels of IFABP have been noted in patients with intestinal necrosis due to obstruction and strangulation versus simple obstruction.[427,430-432] Both surgical trauma and severe sepsis are causes of increased IFABP secondary to mucosal injury, and levels of IFABP are related to the severity of the injury. [431,432] In addition, increased levels of serum IFABP (>355 pg/ml) have been shown to increase the odds of 28-day mortality by 4.46 times in critically ill patients.[433]

In horses with colic, increased IFABP levels measured in abdominal fluid and plasma were positively associated with mortality (P=0.025), and high plasma levels correlated with 4 times higher odds of surgical intervention in horses with colic (P=0.015).[420] Positive correlations were also identified between IFABP and abdominal

fluid protein, age, and plasma aspartate aminotransferase.[420] Interestingly, the highest

levels of IFABP were noted in a horse with a large colon volvulus and peritonitis, which

appears to contradict the known sites for IFABP production in the equine gastrointestinal

tract. The authors suggested hypoperfusion as a reason for the findings, but it is notable

that this horse displayed two known risk factors IAH, which may support the

investigation of IFABP for identification of IAH in the horse.[7,434] While IFABP is

suggested as a marker of ischemic disease in humans, horses demonstrated increased

IFABP secondary to strangulation, supporting its use in this diagnosis. [420]

A multicenter prospective observational study is currently underway in the

Netherlands to assess the levels of IFABP in human patients in intensive care units.[435]

Patients with at least two risk factors for IAH and ACS are included, and IAP will be

monitored with intra-vesicular pressures. Both urine and serum concentrations of IFABP

will be measured with a commercial enzyme-linked immunosorbent assay (ELISA)

selective for human IFABP (HyCult Biotechnology, Uden, The Netherlands). The

prognostic value of IFABP will be assessed, to determine its usefulness as a biomarker of

IAH and ACS, as well as increasing organ dysfunction.

7.2.6   Procalcitonin

Procalcitonin (PCT) is a soluble protein released into the systemic circulation in

response to severe systemic inflammation, particularly bacterial infections.[436] It is the

prohormone of calcitonin, and the equine amino acid sequence contains 115 amino acids

with a molecular weight of 12,507 Da.[437] Phylogenetically, equine calcitonin is placed in

a class with humans, rats and mice on a dendrogram tree, with >87% homology with humans for the amino acid sequence.[437]

The function of PCT as a biologically active molecule is unknown at this time. However, it is classified as a "hormokine" and suggested to have a role in immunomodulation, cytokine expression and vasomotility.[438] The effects of both recombinant and synthetic PCT have been investigated. In vitro, low or moderately elevated levels of PCT were noted to inhibit TNF-α and interferon gamma inducible nitric oxide synthase cDNA production in vascular smooth muscle.[439] However, high concentrations were not inhibitory, and pre-stimulation with PCT resulted in augmentation of nitric oxide production, demonstrating a complicated interaction.[439,440] PCT is also a natural substrate for the immunoregulatory enzyme dipeptidyl-peptidase IV that inactivates various cytokines.[441] Regarding its role modulation of inflammatory mediators, the production of TNF-α can be significantly reduced in the present of either PCT or its c-terminal, 57 amino acid fragment.[442]

Normal PCT levels in humans are <0.1 ng/ml.[443] A reference of 0.2 ng/ml has been recommended as the cut off for exclusion of sepsis and severe inflammation, whereas plasma levels >0.5 ng/ml are interpreted as suggestive of sepsis.[436] After surgical procedures, PCT can increase up to 1 mg/ml to 2 mg/ml depending on the type of surgery.[444] After peaking, circulating levels decline by 50% in 1 to 1.5 days, but may be prolonged with severe renal insufficiency.[436] Half-life of PCT is 18 to 24 hours, and slightly longer at 24 to 30 hours in patients with renal insufficiency.[444] However, the half-life was found in later studies to not be significantly affected by age, gender or renal function.[445]

PCT production outside the parafollicular cells of the thyroid gland is strictly regulated to tissues secondary to stimulation by pro-inflammatory cytokines (e.g. TNF-α, IL-6 and IL-8), and exposure to endotoxins introduced by a variety of injuries including surgery, polytrauma, heat shock, burns, prolonged cardiogenic shock, and severe systemic inflammation from multiple organ dysfunction syndrome.[436,446-451] Induction is stimulated by the activation and adherence of monocytes.[436] However, PCT remains low in other types of inflammation that are not linked to sepsis (e.g. viral infections, autoimmune disease, and organ transplant rejection).[452]

Calcitonin mRNA expression in the horse has been identified in the pituitary gland and the liver, similar to other species.[437,453] Expression in the pituitary gland may be important in regulating cell function.[437] Studies in hamsters have noted PCT production in the lung of non-septic controls, however, when sepsis is induced, low concentrations of PCT can be found in the leukocytes and small intestine, with higher concentrations in the lung, liver, pancreas, colon and other organs.[454] In cell culture, mononuclear cells will express mRNA for PCT if stimulated by endotoxin and proinflammatory mediators.[455] However, they do not play a major role in PCT production, based on clinical observations.[442] In horses, real time PCR was used to investigate gene expression for PCT in the blood of sick and healthy foals, and found no increase in septic foals compared to controls, supporting the finding in humans.[417]

In ischemic events, the release of reactive oxygen species promotes the secretion of inflammatory mediators including TNF-α and IL-6.[456,457] This oxidative stress may increase the permeability of the intestinal mucosa and enterocyte cell gap junctions. Bacterial flora in the gastrointestinal tract may also proliferate if the bowel is ischemic,

generating novel bacterial endotoxins that may cross a leaky gastrointestinal barrier.[458] These endotoxins and inflammatory mediators can result in an increase in the production of PCT and its release into the bloodstream.[459]

The major source of PCT in patients with sepsis or systemic inflammatory response syndrome may be the liver, where high concentrations of PCT mRNA have been identified.[460,461] In a baboon model of hemorrhagic shock, procalcitonin was induced in 4-6 hours after hemoreduction, but to a greater extent in animals in endotoxic shock.[462] If the liver is removed, anhepatic animals did not produce PCT, supporting the role of this organ in sepsis.[463]

Further investigations into the role of PCT in sepsis evaluated purified human PCT from a thyroid medullary carcinoma cell line on both survival and circulation in hamster and porcine endotoxic shock models.[464,465] Survival of hamsters in endotoxic shock was increased significantly if PCT was neutralized, but decreased after PCT infusion.[464] Procalcitonin neutralization also significantly altered hemodynamic parameters in pigs in endotoxic shock,[465] and plasma calcium levels were noted to decrease when PCT was inactivated, similar to patients in septic shock.[454,466,467]

PCT has been recognized as a diagnostic biomarker for sepsis, based on its reliable kinetics for both induction and elimination, allowing a range for unspecified induction to be predicted.[444] After injection of *Escherichia coli* endotoxin in healthy humans, circulating levels of PCT are noted at 4 hours and the level of PCT peaks at 6 hours, remaining stable for 8-24 hours after induction.[468] Circulating levels of PCT are related to the severity of systemic inflammation, and persistently high levels suggest ongoing systemic inflammatory response syndrome or septic complications after

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surgery.[469,470] Both clinical data and preclinical animal studies are in agreement, noting PCT is relevant for diagnosis of ischemia regardless of etiology, with thresholds of 1-2 mg/ml.[457] In horses, a higher basal level of PCT is present in normal individuals, likely due to the presence of large numbers of gram-negative bacteria in the intestinal tract. The presence of these bacteria in the hindgut may allow continuous exposure to small amounts of endotoxin leading to continuous induction of PCT.[415,471] The kinetics after exposure to LPS are similar to humans, with an increase shortly after exposure, and maintenance of increased levels for up to 24 hours.[415]

Procalcitonin is most commonly identified in veterinary and human literature as an early marker of systemic inflammatory response syndrome (SIRS) caused by bacterial infections and associated inflammation.[472] SIRS in adult horses is classified based on the presence of two or more of the following conditions: an abnormal leukocyte count or >10% band neutrophils, hyper- or hypothermia (rectal temperature <37.2°C or >38.3°C), tachycardia (28 to 44 beats per minute), and/or tachypnea (8 to 15 breaths per minute).[473,474] For foals, septic SIRS is noted by leukocytosis or leukopenia (peripheral white cell count >12.5 x 10^9/l, or 10% immature "band" neutrophils), hyper- or hypothermia (rectal temperature >39.2°C or <37.2°C) tachycardia (>120 beats/min), tachypnea (>30 breaths/min), and evidence of sepsis or localized infection.[475] A SIRS scale has been validated for both adult horses and foals.[473,475]

PCT levels have been assessed in both neonatal and adult horses with evidence of SIRS. All studies thus far have noted lower PCT levels in normal versus septic SIRS patients.[474,476] In normal foals between 1-30 days of age, PCT levels ranged from 30 +/-33.1 pg/ml. In comparison, septic foals affected by a variety of conditions, including

colitis, pneumonia, and polyarthritis, demonstrated PCT levels on average of 178.9 +/-
76.1 pg/ml. Based on an ROC curve, the cutoff for septic SIRS in neonates was
established in this group of neonates as 73.04 pg/ml, with 87.5% sensitivity and 97.1%
specificity.[476] In adult horses, a recent study noted significantly higher plasma PCT in
horses with SIRS than healthy horses (197 +/- 117 pg/ml versus 18.28 +/- 20.32
pg/ml).[474] When these horses were subdivided based on the nature of the pathological
process, no difference could be identified between strangulating and non-strangulating
intestinal lesions, or infectious causes of sepsis including colitis and pleuropneumonia.
These values were lower than noted in humans, and higher than that noted in dogs, likely
due to species variation and ELISA differences.[477-479]

Interestingly, PCT may be helpful in identifying patients in a clinical setting that
will not respond to conservative management.[480] In a randomized controlled trial of 166
human patients with small bowel obstructive lesions, PCT levels were higher in patients
where surgical management was needed, and higher in the patients with ischemic lesions
than those found at surgery to have non-ischemic lesions. Procalcitonin was found to
have a significant association with failure of conservative management, as well as
ischemia. Data was validated with a second cohort of 59 patients treated for small bowel
obstruction, and PCT was highest in cases with intestinal ischemic colitis and mesenteric
infarction.[451,457] Overall, the sensitivity of PCT for identification of necrosis and ischemia
based on available evidence in the human literature ranges from 72 to 100%.[451] The
lowest values are for patients with small bowel ischemia (72% to 83%; thresholds of 0.25
to 0.57 ng/ml), whereas the highest are for large bowel ischemia (95 to 100%; threshold

of >2 ng/ml). Clinical data showed a high negative predictive value ranging from 81 to

100% for diagnosis of intestinal ischemia and necrosis.

Chapter 8

Abstract

The recommended technique for abdominal pressure measurement in the horse involves an intra-peritoneal catheter or cannula to obtain pressures directly from the abdominal cavity. This technique is accurate, but invasive, and risks complications including peritonitis, hemorrhage and local abscessation. Biomarkers provide a non-invasive method to diagnose and monitor elevations in intra-abdominal pressures. Abdominal hypertension was created in normal healthy horses, with venous blood samples obtained at set time points over 24 hours during and after the hypertension episode. Serum samples were analyzed by enzyme linked immunoassays specifically designed to evaluate the production of horse interleukin-10, intestinal fatty acid binding protein, and procalcitonin. Analysis demonstrated that the ELISA tests were unable to identify significant changes in any biomarker over the period of the experiment. These results demonstrate that intra-abdominal pressures up to 20 mmHg in healthy horses did not alter biomarkers of ischemia and inflammation.

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8.1 Introduction

Elevated intra-abdominal pressure (IAP) can occur secondary to diseases of the abdominal cavity, systemic inflammation, septic diseases originating outside of the abdominal cavity, or iatrogenic causes including excessive intravenous fluid administration.[7,8] As pressures in the abdominal compartment rise, perfusion of the gastrointestinal organs is reduced by direct compression of both the tissues and vasculature.[41,73] The IAP can also be transmitted across the diaphragm, resulting in reduced pulmonary tidal volumes by interference with movement of the diaphragm.[85] This pressure in the thorax can compress the heart and directly reduce diastolic filling volumes, or indirectly decrease cardiac output secondary to physical compression of the abdominal vena cava.[70-72,75,76] Both local and systemic hypoxia have been identified as causes of organ insufficiency, and eventually multi-organ failure.

In humans, levels of IAP sustained above 12 mmHg are regarded as a disorder called intra-abdominal hypertension (IAH).[7] Sustained IAP greater than 20 mmHg in conjunction with new or progressive organ dysfunction or failure is termed abdominal compartment syndrome (ACS). The incidence of IAH in human patients may range from 31% to 58%.[1] However, for patients with ACS, the mortality rates have been reported at high as 80%.[481] While patients developing ACS may show dramatic and even fatal consequences, lower levels of IAH still cause significant patient morbidity through bacterial translocation across the bowel, ileus, anastomosis breakdown, and wound complications.[41,100,105,110,482]

Horses are similar to humans in regards to the risk factors for increased IAP, and IAH has been identified in horses diagnosed with peritonitis, hepatic abscesses, ileal

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impaction and large colon displacement.[10,15] While indirect methods of IAP measurement used in humans have not translated well to horses due to a lack of accuracy and repeatability, equine IAP can be measured directly using a peritoneal cannula or piezo electric pressure transducer.[11,16] However, the direct method is invasive and risks the development of infection, trauma to the abdominal organs, or fracture of the catheter or cannula within the abdomen. In addition, IAP by itself cannot consistently identify the present of ACS without the concurrent identification of organ dysfunction or failure.[7,8]

Biomarkers are defined as objective methods to assess, diagnose, monitor, or predict and prognosticate disease.[358] Serum biomarkers are by nature minimally invasive, as measurements are performed using samples obtained outside the patient's body. Recently, serum biomarkers have been identified that may allow for identification of the ischemic effects of IAH. These include D-lactate, the N terminal fragments of serum brain-type natriuretic peptide, and adenosine.[373,376,378] However, the application of these biomarkers may be limited for use in the equine species. First, serum D-lactate levels have failed to correlate with the presence of gastrointestinal disease in the horse, although peritoneal levels correlated well, suggesting that serum levels are not appropriate for use of this marker.[374,375] Second, measurement of N terminal fragments of serum brain-type natriuretic peptide is currently not possible in horses, as an equine test does not exist, and the genetic variability of this protein prevents use of a test developed for another species.[377] Finally, adenosine is also not practical as a clinical tool, as measurement employs a reversed-phase high-performance liquid chromatography unit.[378] Therefore, if biomarkers are to be used in the horse, alternative markers will require investigation. There are three that warrant study.

Interleukin-10 (IL-10) is an inhibitory cytokine that modulates the inflammatory response by inhibiting the production of inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) at the level of transcription.[397-401] It can also inhibit cyclooxygenase enzymes and prostaglandin production, as well as increase the proliferation of B-lymphocytes.[397,402] In horses, exposure to lipopolysaccharide has been shown increase production of IL-10 by monocytes within one hour of exposure.[408,415] Serum levels of IL-10 may also serve as a marker of overwhelming infection, as increased levels are associated with a risk of multi-organ failure and death in humans and in horses.[403-405,409,411] In a recent study that evaluated cytokine production in humans with increased IAP, IL-10 was the only cytokine that was significantly elevated by IAH.[378] Although IL-10 has been evaluated horses with gastrointestinal disease, the correlation between IL-10 and IAP in the horse has not been investigated.

Intestinal fatty acid binding protein (IFABP) is a low molecular weight protein found in the cytosol of mature enterocytes at the tips of the mucosal villi of the intestine.[421] This protein is found primarily in the mucosa of small intestine of the horse, and blood levels are typically negligible in the healthy animal.[420] Damage to the enterocytes by trauma or ischemia will release this protein into the bloodstream, and increased levels of serum IFABP has been associated with ischemia, trauma and severe sepsis in human patients.[427-429,431,432] In horses, IFABP levels were highly correlated with the odds of surgical intervention in gastrointestinal disease, supporting the use of IFABP in diagnosis of strangulating lesions in this species.[420] Although IFABP has not been reported in humans as a biomarker of IAH, a prospective study is currently underway to

assess the use of this protein for identification of IAH, ACS and organ dysfunction, as well as prognostication in these diseases.[435] Evaluation of this biomarker in horses in the setting of increase IAP would help to further define the severity and effects of pressure and ischemia on the small intestinal mucosa.

Procalcitonin is a soluble protein that is the prohormone of calcitonin.[436] It is released from a number of tissues in response to inflammatory cytokines, including TNF-α, IL-6 and IL-8, and endotoxins linked to sepsis.[452] A variety of injuries can increase PCT levels in the bloodstream including surgery, polytrauma, heat shock, and severe systemic inflammation due to multiple organ dysfunction.[436,446-451] PCT has a reliable kinetics of induction and elimination in the horse, and therefore is commonly used diagnostic marker for sepsis.[444] In horses, PCT is used as a marker for the systemic inflammatory response syndrome in both foals and adult horses, although PCT was not useful in differentiating the cause of sepsis and inflammation.[474,476] Although the levels of PCT in healthy humans are negligible, <0.1 mg/ml, higher basal levels have been identified in horses, suggested to be due to continuous exposure to endotoxins released from gram negative bacteria involved in hindgut fermentation.[415,443,471] While PCT has not been evaluated in the context of IAH, its current uses would suggest it could be valuable for identification of large intestinal and cecal ischemia.

The purpose of this study was to evaluate the effects IAH on three serum biomarkers, namely IL-10, IFABP, and PCT, in the horse. The hypothesis was that as pressures within the abdomen increase, the levels of these biomarkers would increase over time. Our goal would be to identify a non-invasive, objective measure of abdominal ischemia and compression secondary to elevated abdominal pressures.

8.2 Material and Methods

8.2.1 Animal Selection, Instrumentation, and Insufflation

This study was performed simultaneously with the previous study in Chapter 6. All selection, instrumentation and procedures are described in section 6.2. Only the additional techniques for measurement of biomarkers are described herein.

8.2.2 Phlebotomy Procedure

Blood was obtained for measurement of serum biomarkers using direct stick of the left jugular vein, ventral to the jugular catheter present in the left jugular vein. Blood was collected using a vacutainer system into plain borosilicate tubes without anticoagulant at the following timepoints during insufflation: baseline, 30 minutes, 40 minutes, 50 minutes, and at 60 minutes. All samples during the experiment after the baseline were obtained at an abdominal pressure of 20 mmHg. Additional blood samples were collected at 1, 2, 4, 8 and 12 hours after deflation of the abdomen. Samples were allowed to clot at room temperature (21°C) for 30 minutes, and then centrifuged at 2000 rpm for 10 minutes to collect the serum. The serum was allocated into 1 ml Eppendorf tubes, and frozen immediately at -80°C for future batch analysis.

8.2.3 Biomarker Analysis

Biomarkers were measured using commercial quantitative sandwich ELISA kits per the manufacturer's instructions. The serum was thawed at room temperature (21ºC), and no sample was subjected to repeat freeze-thaw cycle.

8.2.3.1 Interleukin-10 Analysis

For measurement of IL-10, a commercial ELISA was used (horse interleukin-10 ELISA kit, catalog number MBS280686, MyBioSource, Inc., San Diego, CA). Briefly, a total of 100 microliters of serum were pipetted into separate microelisa stripplate wells coated with antibody for horse procalcitonin. All samples were assayed in duplicate. The standards for procalcitonin were also diluted and run in duplicate on the same plate. The plate was incubated for 2 hours at 37ºC. The plate was washed three times using 250 microliters wash buffer per well per wash with an automated plate wash system, and blotted dry. A total of 100 microliters of the biotin-conjugate was added to each well to bind to bound IL-10, and the plate was incubated for 1 hour at 37ºC. The plate was washed three times using 250 microliters wash buffer per well per wash with an automated plate wash system, and blotted dry. Next, a total of 100 microliters of horseradish peroxidase-streptavidin regent was added to each well, and the plate incubated for one hour at 37ºC. The plate was washed three times using 250 microliters wash buffer per well per wash with an automated plate wash system, and blotted dry. The substrate solution was added in aliquots of 100 microliters, mixed, and protected from light during a 15-minute incubation at 37ºC. At this time, 50 microliters of stop solution was added to each well to cease the reaction, the wells mixed, and the optical density of each well of the plate was read at 450 nm with an electronic ELISA plate

reader. The sensitivity of the kit was 7.8 pg/ml; with a detection range of 15.6 pg/ml to 1000 pg/ml. Reproducibility was identified by the manufacturer through tests of intra-assay and inter-assay precision. The intra-assay coefficient of variation was <8% and the inter-assay coefficient of variation was <12%. Cross-reactivity or interference with analogues of horse IL-10 has not been observed by the manufacturer.

8.2.3.2 Intestinal Fatty Acid Binding Protein Analysis

For measurement of IFABP, a commercial ELISA was used (horse intestinal fatty acid binding protein ELISA kit, catalog number MBS024340, MyBioSource, Inc., San Diego, CA). Briefly, a total of 50 microliters of serum were pipetted into separate microelisa stripplate wells coated with antibody for horse IFABP. All samples were assayed in duplicate. The standards for equine IFABP were also run in duplicate on the same plate. A total of 100 microliters of the horseradish peroxidase-conjugate regent was added to each well to bind to the bound horse IFABP, and the plate incubated for one hour at 37°C. The plate was washed four times using 350 microliters wash buffer per well per wash with an automated plate wash system, and blotted dry. The chromogen solutions (50 microliters A and 50 microliters B) were added in succession, mixed, and protected from light during a 15-minute incubation at 37°C. At this time, 50 microliters of stop solution was added to each well to cease the reaction, and the optical density of each well of the plate was read at 450 nm with an electronic ELISA plate reader. The sensitivity of the kit was 10 pg/ml, with a detection range of 62.5 pg/ml to 2000 pg/ml. Intra-assay and inter-assay reproducibility were identified by the manufacturer as a coefficient of variation of <15%.

8.2.3.3 Procalcitonin Analysis

For PCT measurement, a commercial ELISA was used (horse procalcitonin ELISA kit, catalog #MBS041197, MyBioSource, Inc., San Diego, CA).   Briefly, 50 microliters of serum were pipetted into separate microelisa stripplate wells coated with antibody for horse PCT. All samples were assayed in duplicate. The standards for equine PCT were also diluted and run in duplicate on the same plate. A total of 100 microliters of the horseradish peroxidase-conjugate regent was added to each well to bind to the bound horse procalcitonin, and the plate incubated for one hour at 37ºC. The plate was washed four times using 350 microliters wash buffer per well per wash with an automated plate wash system, and blotted dry. The chromogen solutions (50 microliters A and 50 microliters B) were added in succession, mixed, and protected from light during a 15-minute incubation at 37ºC. At this time, 50 microliters of stop solution was added to each well to cease the reaction, and the optical density of each well of the plate was read at 450 nm with an electronic ELISA plate reader. The sensitivity of the kit was 10 pg/ml, with a detection range of 50 pg/ml to 1600 pg/ml. Intra-assay and inter-assay reproducibility were identified by the manufacturer as a coefficient of variation of <15%.

8.2.4   Statistical analysis

Data was collected and collated using commercial software (Excel, Microsoft Windows 2010, Microsoft, Inc., Redmond, WA). For each ELISA, the standard curve was determined after adjusting each absorbance by subtracting the absorbance of the blank. Regression analysis was performed to obtain the standard curves, and a Pearson's

product moment correlation performed to determine correlation and significance. The regression curves were used to calculate the concentration of the biomarker in each sample. Samples with concentrations less than 0 were determined to be undetectable by the test kit, and were reported as 0 pg/ml. Homoscedasticity was evaluated using a Bartlett's test for comparison of data, and inspection of boxplots of the data. For data with normal distributions, comparisons were performed with a one-way analysis of variance, and significant differences were further analyzed with post hoc testing when appropriate. A value of P<0.05 was considered significant. Statistical analysis was performed using commercial statistical software (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

8.3   Results

8.3.1   Interleukin 10 Production

Interleukin 10 was identified in horses at all time points. The regression lines for the samples demonstrated fair to good correlation (r^2: 0.529, P=0.101; r^2: 0.877, P=0.0006067). Data was normally distributed, and the serum concentrations of IL-10 were not statistically different for any timepoint during the experiment (P=0.05912). (Figure 8-1)

8.3.2   Intestinal Fatty Acid Binding Protein Production

Intestinal fatty acid binding protein (IFABP) was identified in samples at all time points. The regression lines for the samples demonstrated excellent correlation (r^2: 0.996, P=2.366e-06; r^2: 0.993, P=7.128e-06). Data was normally distributed, and the serum concentrations of IFABP were not statistically different for any timepoint during the experiment (P=0.4342). (Figure 8-2)

5.3.3 Procalcitonin Production

Procalcitonin (PCT) was identified in samples at all time points. The regression lines for the samples demonstrated excellent correlation (r^2: 0.904, P=0.001004; r^2: 0.949, P=0.000199). Data was normally distributed, and the serum concentrations of PCT were not statistically different for any timepoint during the experiment (P=0.4174). (Figure 8-3)

5.4 Discussion

Serum concentrations for IL-10, IFABP, and PCT were detectable the horses at all timepoints during the study using a commercial ELISA. However, a trend was not appreciable for any of these biomarkers to show temporal production or correlation after a period of abdominal insufflation up to 20 mmHg in standing horses.

Serum levels of IL-10 are known to increase in the presence of endotoxemia, sepsis and septic shock.[403-405] In addition, gene expression for IL-10 increases quickly in the present of inflammation caused by lipopolysaccharide.[409,411,417,483] The kinetics of IL-10 production in the bloodstream in the horse is faster than humans, with levels increasing within one hour of exposure to the inciting cause.[415,484] Therefore, we expected

to see IL-10 production rise within an hour after insufflation. However, IL-10 was already present in the samples at baseline, indicating that possible exposure to endotoxin or tissue trauma had already occurred. A possible source was unknown. A second explanation for baseline production could be inaccuracies in the ELISA itself, as the concentrations of IL-10 reported in this study were highly variable and the fact that the test was not independently validated. Although high variability was noted in humans when assessing the effect of increasing IAP, the concentrations were much higher than those reported in humans with and without elevated IAP.[378] In normal horses in other studies, IL-10 concentrations were still lower than the horses in our experiment, averaging around 37 pg/ml at baseline.[415] (Table 8-1) Even after exposure to LPS, levels in these horses quickly increased at one hour, but on average did not exceed 100 pg/ml.[415] Although the ELISAs used were different between the previous study and ours, the antibodies used in both assays were equine, therefore cross-reaction is unlikely. Again, a fault may lie in the ELISA process or methods, as the results were inconsistent with other reports.

In this report, IFABP was detectible at all timepoints, but not in all horses at each timepoint. Previous work has demonstrated that concentrations of IFABP are undetectable in the bloodstream of healthy horses and humans.[420,422] (Table 8-1) The source of IFABP in the horse is the small intestine, primarily the jejunum, followed by the ileum and duodenum.[420] Detection of IFABP in the bloodstream should indicate acute injury to the villi, as the concentrations of the protein are finite and depleted quickly.[423] As there was no evidence of intestinal trauma or colic prior to or after the experiment, the source of IFABP is unknown. A simple explanation may be the differences in test kits.

The previous reports used an ELISA with antibodies produced against human IFABP, whereas an equine specific antibody was used in the present study. Although IFABP structure and amino acid number are highly conserved between species, variations in the antibody as well as manufacturer's process may account for the differences seen.[420,485] A previous study found IFABP was also not sensitive for detecting ischemia in horses with colic; therefore, it may not serve well as a biomarker for IAP in early detection of early ischemic disease.[420] (Table 8-1)

Concentrations of PCT measured in horses in this study were much higher than previously reported. (Table 5-1) In the current study, the samples obtained from the horses at time 0 had a mean PCT concentration of 454.0 pg/ml (standard deviation 175.1 pg/ml), which was more than 25 times that of healthy normal controls in a previous report using an identical ELISA.[474] In comparison with horses with SIRS, as well as horses exposed to endotoxin, the levels in this study were still much higher.[415,474] (Table 8-1) Although higher levels have been reported in septic horses, the differences in that report can be attributed to an ELISA developed for equine PCT, which used antibodies targeted for human PCT.[486] While the molecules are similar, the horse PCT molecule is only 74% homologous with human PCT.[437] In addition, the working range of the test used by Reiger et. al. was from 25 to 1000 ng/ml, and it will not register smaller concentrations, as seen in foal and adult cases of SIRS.[474,476] As recent studies have discovered a positive background level of PCT, the range of this ELISA in Reiger et. al. is likely skewed.[415] In the present investigation, the ELISA kit used was identical to the one in Bonelli et. al., which was an equine ELISA kit with antibodies specific for this species, therefore the

results should have been comparable.[415,474] Defects in the kits or shipping processes may also be an explanation for the discrepancy.

As levels for all biomarkers were already increased at time 0 prior to insufflation, it is possible that either the horses were exposed to a bacterial infection or endotoxin prior to the study, or the trauma of instrumentation may have exposed the horses to endotoxin or inflammation that increased the levels of the biomarkers measured. Endotoxins may also have been present in the medications or fluids administered to the horses.[487] A control group with the same husbandry conditions would have helped to identify the effects of instrumentation compared to preexisting conditions. Unfortunately, a complete blood count, biochemical analysis, serum lactate or amyloid A testing was not performed to assess a preexisting systemic reaction to infection or inflammation; therefore, it is not possible to speculate. Improved screening of the individual horses included in the study may be needed to explain or eliminate the unusual results noted at baseline in this study. In addition, a sample obtained prior to instrumentation may identify any changes caused by an acute inflammatory reaction to catheterization. However, all assays did perform well in regards to the manufacturer's standards for the equine biomarkers, so inaccuracies in the ELISA itself are less likely. Further validation with known laboratory standards and evaluation of the performance of the ELISA compared to blank equine serum samples may improve the interpretation and confidence in the results.

It is important to note that PCT can undergo enzymatic cleavage, and that the fragments can be identified in plasma.[438,456,488] These include an N-terminal aminoprocalcitonin fragment and calcitonin:katacalcin fragment, which is also measured

as PCT by commercial assays. These fragments can be found in both plasma and septic tissues, and may be in comparable levels to the concentrations of the whole molecule. Therefore, the levels measured may include both whole PCT and its byproduct.

In humans, clinical measurement of PCT is conducted using patented kits that use either an immunoluminometric assay, (ILMA LUMItestRPCT, Thermo Fisher Scientific, Inc.), an assay based on time resolved amplifies cryptate emission (TRACE) technology, or a semi-quantitative rapid test (BRAHMS PCT, Thermo Fisher Scientific, Inc.).[436] The LUMItestRPCT uses mouse monoclonal antibodies to human calcitonin and katacalcin. Krypto rPCT is a sheep polyclonal anti-calcitonin antibody and a monoclonal anti-katacalcin antibody. Neither the N terminal amino acids (aminoprocalcitonin) nor the amino acids distinguishing PCT-I and PCT-II are required for antibody binding. These assays measure PCT-I and -II and various cleavage products containing the residues of calcitonin and katacalcin. It has been highlighted in human literature that different research methods may identify different concentrations for different calcitonin precursors, and different ranges.[452] The test used should be consistent and taken into consideration when identifying cutoffs for diagnosis and prognosis.

Limitations of this study are the low numbers of animals (number = 8). A larger study population would be needed to identify the effect of IAP on these biomarkers in clinical cases, as the variation was large for all three markers. However, the variation noted in this study, as well as previous investigations, may prevent these markers from providing any useful information to distinguish effects of ischemia or inflammation. Further investigation using a study design with both control horses and horses exposed to IAH will also be needed to remove background inflammation and determine the effects

of instrumentation on biomarker levels. Based on post-hoc testing using results for PCT obtained by Bonelli et. al., a total of 16 horses would be needed with a power of 0.9, and an α (type I error) level of 5%.[415] It would also be prudent to compare and cross reference the samples obtained with ELISA tests from other companies, as there may be issues with consistency across platforms.

In conclusion, the use of IL-10, IFABP, and PCT as biomarkers for assessment of the effects of increased IAP cannot be recommended at this time, with the ELISA kits used to perform measurement in this study, without further validation.

Table 8-1 Comparison of biomarker levels obtained in the present study compared to previous experiments in both humans and horses. The concentrations are listed as reported in each study. The average concentrations of all biomarkers were higher in the current investigation than previously reported for both normal, healthy individuals as well as those stimulated by lipopolysaccharide or naturally occurring disease processes.

INTERLEUKIN 10 (IL-10)								
						hours after stimulus		
experiment	test used	comparison	units	group	baseline	1	2	24
Present study	Commercial ELISA (MyBioSource, Inc., San Diego, CA)	Healthy horses stimulated with increased IAP (acted as own controls)	pg/ml		715.3+/-403.4	462.3+/-580.0	671.7+/-468.2	990.6+/-838.1
Bonelli et. al. 2017[415]	fluorescent bead-based assay, equine antibodies	Healthy horses stimulated with LPS (acted as own controls)	(measured in median florescence intensities)		31.2+/-15.6	97.4+/-67.8	97.3+/-54.4	33.2+/-19.7
Burton et. al., 2009[416]	Fluorescent bead-based assay, equine antibodies	Healthy foals compared to septic foals, commercial plasma and mares	pg/ml	Healthy foals	<4			<4
				Septic foals	Approx. 50			<4
				Commercial plasma	0			
				Mare serum, with healthy foal	<4 (range <4-172)			
				Mare serum, with septic foal	<4 (range <4-963)			
Bodnar et. al., 2010[378]	Commercial ELISA (Pharmingen, Inc.)	Humans with and without IAH	pg/ml	Humans with IAH	63.23 +/-58.41			
				Humans without IAH	27.27 +/-58.41			

INTESTINAL FATTY ACID BINDING PROTEIN (IFABP)								
						hours after stimulus		
experiment	test used	comparison	units	group	baseline	1	2	24
Present study		Healthy horses stimulated with increased IAP (acted as own controls)	pg/ml		305.6+/-217.3	420.1+/-446.4	401.1+/-393.1	398.9+/-254.8
Nieto et. al., 2005[420]	Commercial Human IFABP ELISA (HyCult Biotechnology, Uden, The Netherlands)	Healthy horses			<limit detection			

PROCALCITONIN (PCT)								
						hours after stimulus		
experiment	test used	comparison	units	group	baseline	1	2	24
Present study	Commercial ELISA (MyBioSource, Inc., San Diego, CA)	Healthy horses stimulated with increased IAP (acted as own controls)	pg/ml		267.1+/-207.1	246.5+/-228.3	310.7+/-294.8	300.1+/-294.9
Bonelli et. al. 2017[415]	Commercial ELISA (MyBioSource, Inc., San Diego, CA)	Healthy horses stimulated with LPS (acted as own controls)	pg/ml		28.5+/-23.3	193.0+/-144.5	219.1+/-143.9	271.0+/-131.9
Bonelli et. al. 2015[476]	Commercial ELISA (MyBioSource, Inc., San Diego, CA)	Healthy foals compared to foals with SIRS	pg/ml	Healthy foals	30.0+/-33.1			
				Septic foals with SIRS	178 +/-76.1			
Bonelli et. al. 2015[474]	Commercial ELISA (MyBioSource, Inc., San Diego, CA)	Healthy horses compared to horses with SIRS	pg/ml	Healthy horses	18.28+/-20.32			
				Horses with SIRS	197.0+/-117.0			

Figure 8-1 Boxplot (median, 25th and 75th quartiles, range) of comparisons of interleukin 10 (IL-10) in pg/ml over time in minutes. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg for 30 minutes. Pressures for the remainder of the experiment were not artificially elevated. Concentrations of IL-10 were not significantly different for any timepoint (P=0.05912).

Comparison of IL-10 Concentrations over Time
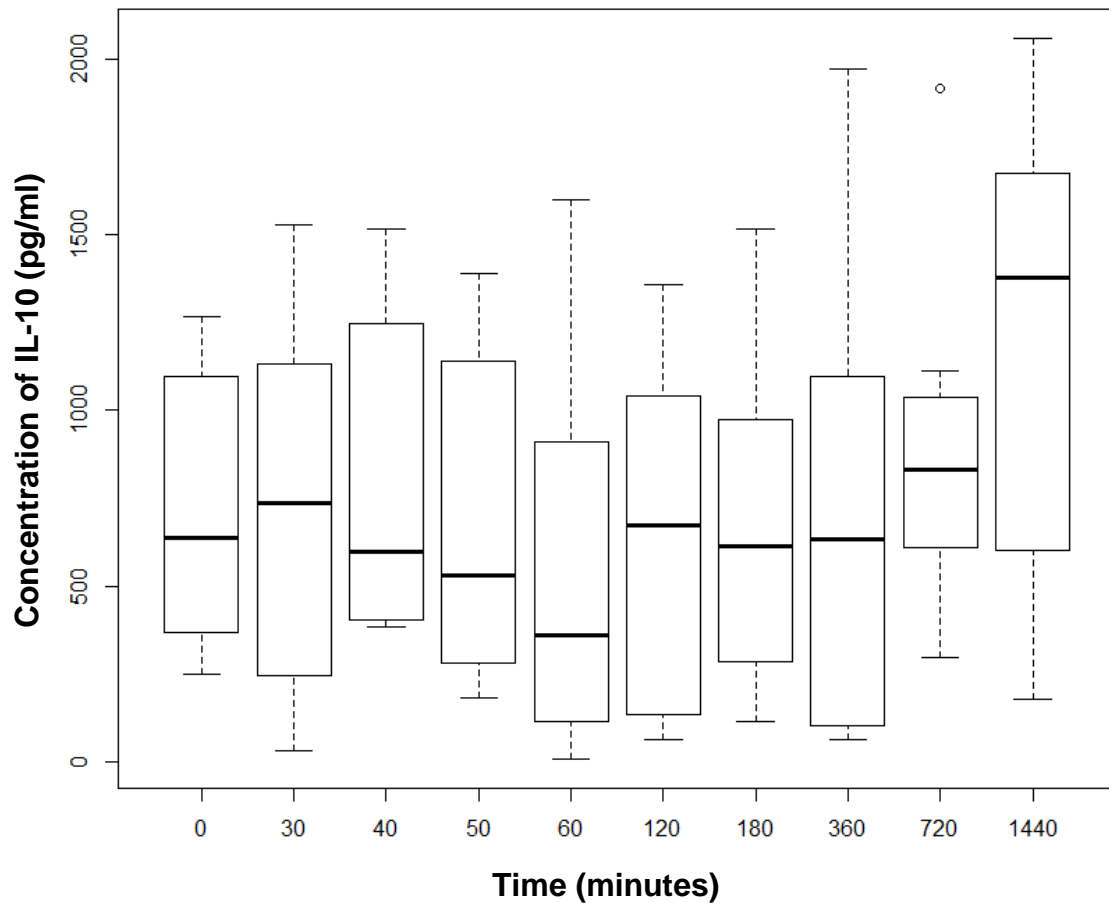
Figure 8-2 Boxplot (median, 25th and 75th quartiles, range) of comparisons of intestinal fatty acid binding protein (IFABP) in pg/ml over time in minutes. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg for 30 minutes. Pressures for the remainder of the experiment were not artificially elevated. IFABP was not significantly different for any timepoint (P=0.4342).

Comparison of IFABP Concentrations over Time
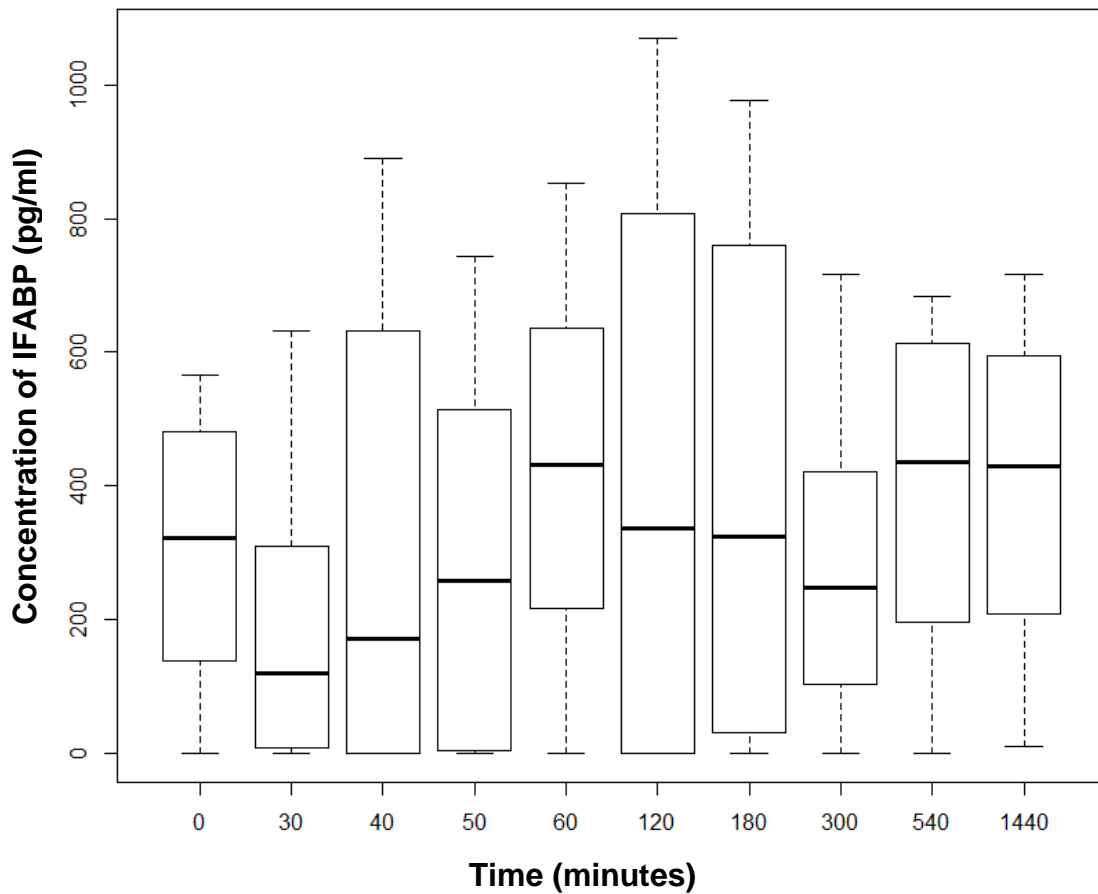
Figure 8-3 Boxplot (median, 25th and 75th quartiles, range) of comparisons of procalcitonin (PCT) in pg/ml over time in minutes. Pressures in the abdomen increased in a stepwise fashion until 30 minutes, when pressures were maintained for 30 minutes at 20 mmHg for 30 minutes. Pressures for the remainder of the experiment were not artificially elevated. PCT was not significantly different for any timepoint (P=0.4174).

Comparison of PCT Concentrations over Time
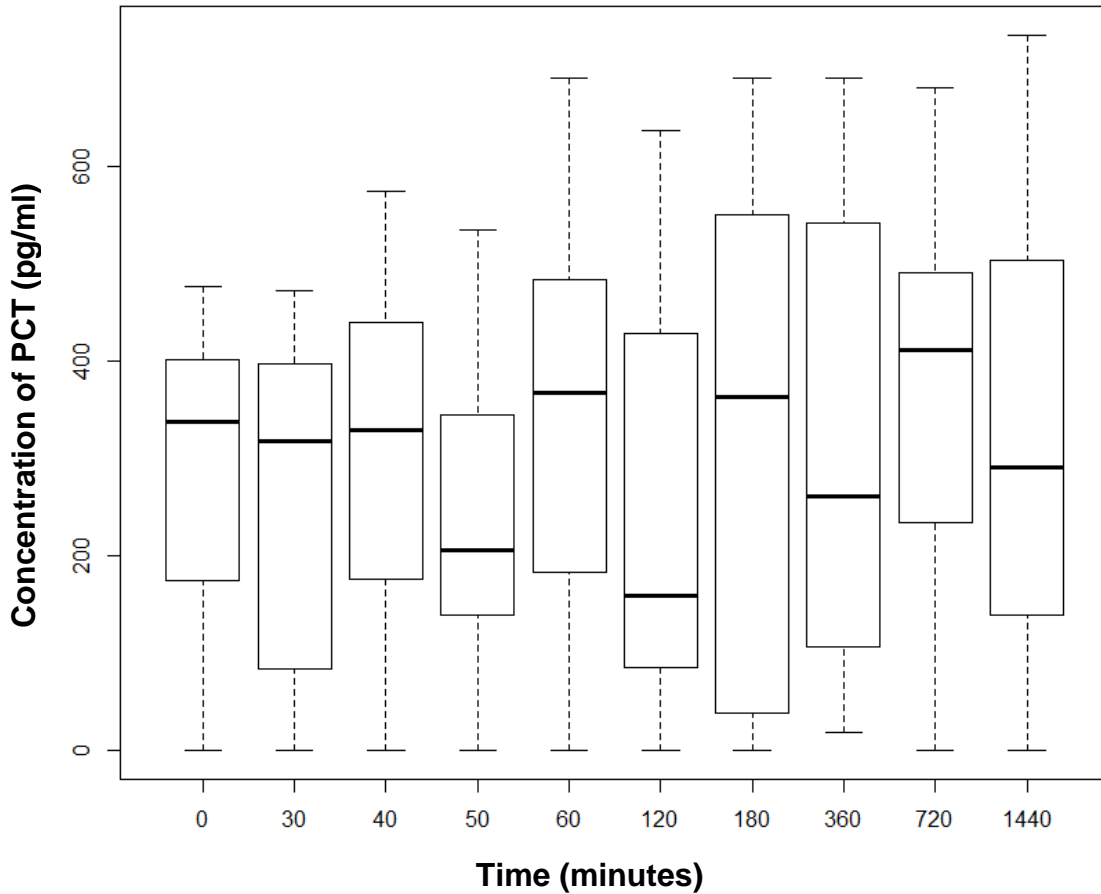
Chapter 9

9.1 Introduction

The purpose of the research investigations pursued in the course of this dissertation were to determine a method of accurate and minimally invasive identification of intra-abdominal pressure (IAP) in the standing horse. Initial work identified the ability to measure the IAP from the abdominal cavity using direct peritoneal puncture.[11,12,16] However, this method for measurement of IAP was not readily accepted or implemented in clinical cases, due to its invasiveness and the risk (albeit minimal) of peritonitis, organ damage, and subcutaneous infection. This led to further investigations into less invasive, indirect methods of IAP measurement derived from methods utilized in human medicine, including intra-vesicular and intra-gastric pressure measurement. Unfortunately, there was limited success in transferring these methods from humans to horses.[11,12] The lack of correlation between these techniques could be contributed to anatomical and species differences, as well as the difference in patient management between standing, awake horses and supine, sedated humans in the ICU.

The results of continued investigations in this field have determined that the pressures obtained by a femoral venous catheter can be used to determine the pressure within the abdomen in the horse, and are accurate with a correction factor to account for

gravitational forces. This catheter is less invasive than the previously validated peritoneal catheter, and should be readily accepted by the veterinary community, as it requires no additional specialized equipment. The femoral venous catheter may also be used to obtain continuous pressure measurements, and is much more cost effective than the previously validated piezo electric probe used for this purpose.[16] This catheter can also provide additional functions, including the administration of intravenous medications, fluids and can allow clinicians to obtain blood samples for biochemical analysis. Femoral venous pressure measurement as a measure of IAP provides a simple methodology to advance research in this field in clinical equine patients.

9.2   Measurement of Intra-Abdominal Pressure in Clinical Cases

In veterinary medicine, the approach to identification and treatment of compartment syndrome of the abdomen is still in its infancy. At this stage, clinicians are working to determine the best methodology for obtaining IAP in equine patients, and a validated scale of severity for these pressure measurements has yet to be identified. It is known that clinical examination is not useful in determining IAH; therefore, direct methods for measurement are required. As femoral pressure measurements appear promising, a future goal is to establish a scale of severity for the levels of IAP recognized in clinical equine patients. Once able to classify IAP in relation to morbidity, complications, and mortality, clinicians will be able to better understand how IAP can guide the decision making process in regards to interventions and therapy of the diseases that cause IAH in horses.

Diseases and disorders known to increase IAP in horses include large intestinal displacements, large intestinal volvulus, uterine hydrops, peritonitis, and liver disease, and many of the risk factors for intra-abdominal hypertension (IAH) in humans cross species lines.[7,10,201] It is known that as pressure increases in the abdomen, the perfusion of the abdominal organs declines due to compression of the tissues as well as reductions in cardiac output caused by diminished venous return from the caudal venal cava.[8] Consequences of tissue ischemia secondary to IAH include bacterial translocation, sepsis, renal insufficiency, hepatic necrosis, and diminished wound healing.[82] In humans, recognition of an elevated IAP in a patient sets in motion a series of interventions used to reduce the pressure in the abdomen and improve tissue oxygenation. Among these are fluid evacuation from the peritoneal space, gastric and colonic lavage and decompression, and abdominal exploration to correct obstructions and distention of the gastrointestinal viscera.[7] Evidence that these risk factors for IAH cross species lines make measurement of IAP a necessity for the evaluation and treatment of critically ill horses. While medications can be used for management of lower grades of IAH, surgical intervention is required for advanced stages and patients with abdominal compartment syndrome.

Clinical cases have provided evidence that the presence of IAH can negatively affect the outcome of disease processes in the horse. Brosnahan et. al. presented two equine cases with IAH secondary to peritonitis, and both were euthanized secondary to a perceived poor prognosis by the treating clinicians.[10] In these horses, perfusion parameters remained negatively affected by the increased IAP, despite what was perceived as adequate fluid resuscitation. The question that still must be answered is whether paracentesis to treat IAH will improve cardiovascular perfusion in horses with

peritoneal effusions, and whether decompression may have improved the clinical outcome in these horses. Further investigations in cases of peritonitis would be required, with serial monitoring of IAP as well as therapeutic management of both the infection and IAH to determine the effect on outcomes and recommendations for therapy.

Measurement of IAP will also be helpful to guide surgical intervention in horses with gastrointestinal disease, especially in cases where financial obligations limit therapy. An example of this is a case of large intestinal displacement and impaction that presented to Auburn University with a high heart rate (>80 beats per minute), but a normal peritoneal tap. During the course of therapy, the horse began to produce significant amounts of positive net reflux, up to 10 liters every 4 hours. Abdominal pressures were initially measured at approximately 5 mmHg. However, abdominal pressures dropped to normal levels (subatmospheric) after 12 hours of medical management, coinciding with cessation of colic signs. This was despite the fact that the horse continued to produce net gastric reflux and maintained a heart rate above 80 beats per minute for more than 8 hours after resolution of IAH. As vital parameters and signs of obstruction were slower to resolve, IAP appears to be an earlier indication of resolution of intestinal obstruction or displacements than currently used clinical observations.

Similar findings were observed in a second horse presented to the University for a presumed ileal impaction, where initial IAP measurements were above 20 mmHg. The IAP decreased to normal ranges before signs of small intestinal obstruction resolved, similar to what was described for the horse with the large colon displacement. In addition to therapeutic monitoring, persistent signs of pain, along with elevated IAP may provide evidence that surgical decompression is needed. This was demonstrated in a third horse

diagnosed with a right dorsal displacement, where persistent abdominal pain and elevated IAP provided evidence for surgical intervention. Similar observations are noted in human medicine, and the combination of abdominal pain and IAH are identified as consistent markers of a surgical lesion.[489]

9.3   Role of Abdominal Compliance in Interpretation of Intra-Abdominal Pressures

Future investigations will need to define the IAP measurements consistent with IAH in traumatic injury, surgical lesions, as well as medical disease in veterinary medicine. However, IAP is not the sole factor that determines the development of IAH and abdominal compartment syndrome. In humans, the relationship between abdominal volume and abdominal pressure is described by abdominal compliance.[33] Abdominal compliance is determined by the ability of the abdominal wall to expand, as well as the elasticity of the abdominal wall and diaphragm.[7] Abdominal compliance is calculated by the change in intra-abdominal volume (IAV) divided by the change in IAP.[33]

The relationship between IAV and IAP is linear at pressures lower than 15 mmHg. As pressures increase in the abdominal cavity, the abdominal pressure and volume relationship will eventually reach a point where a small change in volume will produce an exponential increase in pressure within the abdomen as the compliance of the abdominal cavity is exceeded.[33] While abdominal pressure can be measured, IAV is often an unknown factor in horses. Volume has been determined in human patients, estimated around 13 L in normal, healthy adults, but baseline levels are not yet identified in horses.[40] Future investigations may provide evidence for the normal range for IAV in horses, to further define its effects on IAP, abdominal compliance, and the development

of IAH. Abdominal circumference may be useful to identify abdominal volume.[121] However, complicated indexes may be needed to account for intra-abdominal adiposity.[490]

Alternatively, measured changes in abdominal pressure and volume within the abdomen could be used to identify the compliance of the equine abdominal cavity without knowing the specific volume of the cavity. Measurement of the volume of CO_2 insufflated into the cavity could be directly measured during laparoscopic procedures to identify volume, but would not be effective for clinical monitoring.[491,492] Changes in volume could also be identified with the addition or removal of gastric contents, and our research group has previously validated this technique in healthy horses.[39,233] In clinical cases, the removal of peritoneal fluid and the corresponding change in IAP could be used to calculate the compliance of the equine abdomen.[40,194] These methods require further investigation.

While investigations have focused previously on only IAP to determine the present or absence of IAH, the compliance of the abdomen can provide significant information to determine if patients will be more likely to develop complications from rapid increases in IAP with changes in IAV. Studies will be needed in horses to identify accurate and clinically relevant methods for measurement of abdominal compliance. In addition, the human risk factors for decreased abdominal compliance cited in Chapter 1 should also be identified for relevance in equine patients, alongside the risk factors for IAH, to determine those at risk of developing this disease.

9.4   Investigations in Therapeutic Interventions for Intra-Abdominal Pressure

Current methods for treatment of IAH in humans include reductions in intra-abdominal volume or improvements in intra-abdominal compliance.[7] As the ability to measure IAP in horses becomes more commonplace, it will be interesting to observe if commonly used therapies for human patients will effect changes in IAP in this species as well. Therapeutic interventions include methods to reduce IAV or increase intra-abdominal compliance to reduce IAP.

9.4.1   Evaluation of Therapies to Reduce Intra-Abdominal Volume

Treatments to reduce IAV include gastric decompression with a nasogastric tube, prokinetics, paracentesis for relief of abdominal effusion, and surgical decompression.[33] Many cases of equine gastrointestinal disease have large volumes of free fluid within the abdomen; the ability to monitor changes in IAP may further investigations to determine if decompression improves clinical outcomes. IAP measurement may be used in horses to provide more targeted fluid administration, guide the use of colloid administration, and reduce organ edema.[493] However, when medical therapy fails to alter IAP, surgical decompression is still recommended based on experiences in human medicine. Exploratory laparotomy has been observed to reduce IAP in the equine case of large colon displacement previously mentioned; it is also useful for surgical conditions in other veterinary species, including gastric dilatation and volvulus and pyometra in dogs.[142,145] Measurement of IAP in horses will help to establish guidelines for clinicians when surgical intervention is needed.[494]

9.4.2   Evaluation of Therapies to Improve Abdominal Compliance

Abdominal compliance may be more difficult to improve in equine patients. Adequate sedation and analgesia are recommended in humans to reduce abdominal splinting and muscle tone to increase compliance.[33] However, it may be difficult to obtain abdominal wall relaxation in horses that are awake and standing, compared to humans under strong sedation or chemically paralyzed in supine recumbency. Abdominal bandages are also contraindicated in human patients at risk for IAH, but that recommendation for horses is currently unclear.[33] Bandages are commonly used to protect and provide compression to celiotomy incisions, and may reduce the incidence of incisional infections.[495] Conversely, elevations in IAP caused by these bandages may reduce tissue healing by decreasing tissue perfusion, and increase the risk of herniation.[110] A study in normal horses by our research group observed that abdominal compression bandages increased IAP significantly compared to baseline.[496] Further investigations will be needed to determine the effects of abdominal bandages in horses with alterations in abdominal compliance, and to evaluate the effects on incisional healing in horses with increase IAP.

9.4.3 Novel Therapies for Equine Intra-Abdominal Hypertension

As the technique for femoral venous pressure measurement will now provide a way to monitor IAP in horses, it will also provide a way to examine the effects of novel therapies for IAH in this species. One such therapeutic is theophylline. Theophylline is a weak, non-selective phosphodiesterase inhibitor. At high concentrations, it results in an increase in cAMP by inhibition of phosphodiesterase 3 and 4. It can also exhibit its effects through cyclic guanosine 3'5'-monophosphate, with inhibition of

phosphodiesterase 5.[497] Inhibition of phosphodiesterase enzymes can attenuate production of superoxide radicals, leukotriene B_4 synthesis, degranulation, and chemokine release by equine neutrophils.[498] At lower concentrations, theophylline continues to demonstrate anti-inflammatory effects, reducing neutrophil chemotaxis and IL-8 production, however, the mechanism remains uncertain.[499] There is some evidence that low dose theophylline can prevent translocation of the transcription factor nuclear factor-kappaβ to the nucleus, inhibiting the initiation of inflammatory gene expression directly.[500] Theophylline may also activate histone deacetylase enzymes, interfering with the physical unwinding of DNA for the transcription of these inflammatory genes.[501]

Theophylline is a potent inhibitor of both the A_1 and A_2 adenosine receptors, with some effects on the A_3 receptor.[502] In this capacity, it has been investigated in regards to its renal-protective effect. The adenosine receptors that promote vasoconstriction of the renal arteriole have been shown to be susceptible to antagonism by theophylline.[503] In human patients administered cisplatin, a nephrotoxic chemotherapeutic agent, theophylline has demonstrated the ability to preserve renal function when administered before and during the infusion of the drug.[504] Similar results have been noted in clinical studies where other nephrotoxic agents have been administered, including radiocontrast media.[505]

In horses, theophylline is mainly administered for adjunctive therapy of recurrent airway obstruction.[506,507] Pharmacokinetic data indicates that systemic doses of 9 to 15 mg/kg can produce serum concentrations between 10 and 20 μg/kg within an hour after administration.[506] Signs of toxicity in humans may occur at doses greater that 20 mg/kg, including nausea, seizures and cardiac arrhythmias; however, horses have not shown any

of these adverse reactions in pharmacokinetic evaluations of the drug.[506,508] The range for anti-inflammatory effects in other species is between 5 and 10 µg/kg, and a steady state of 10 µg/kg has been shown in the horse after administration of 6.2 mg/kg bid.[506] At this time, there are no studies that have shown the effects of theophylline on renal function in horses. Theophylline also has not been evaluated in the face of elevations in IAP in the horse. Further investigations into the effect of theophylline infusions in ameliorating the negative consequences of IAH on tissue perfusion is needed in horses, or through a rabbit model of this disease that is currently being developed.

Conclusions and Summary

Initial investigations in normal, healthy horses demonstrated that IAP could be measured directly from the peritoneal cavity using a metal cannula and water manometer. Indirect methods of IAP measurement, which measure pressure transduced into the fluid contained within a hollow organ with in the abdomen as a surrogate for IAP measurements, would be preferred, as they are less invasive. Measurements of gastric and urinary bladder pressures as indirect measures of IAP in healthy, standing horses were found to be highly variable, and did not correlate with pressures obtained from the peritoneal cavity. Based on these findings, direct abdominal puncture was determined to be the recommended method for IAP measurement in the horse. An additional study validated a piezo electric solid-state microsensor pressure transducer to allow for continuous, direct measurement of IAP.

Further investigations were performed to evaluate alternative methods for measurement of IAP in the horse using vascular pressures, including CVP and FVP. CVP measurements did not correlate directly with increasing IAP in standing, healthy horses. There was a trend for a positive correlation with IAP up to 12 mmHg, and a negative correlation as pressures continued to rise; however, the large variance prevented assessment statistical significance of these observations with the small number of horses sampled. A strong correlation was observed between FVP and pressures obtained simultaneously from within peritoneal cavity. Femoral pressures were influenced by gravity and the distance from the heart in the standing horse, and were significantly more

positive than pressures obtained from the abdomen. A correction factor was determined to allow for conversion between FVP and direct IAP. The correlation of the FVP with insufflation pressures was improved compared to the previously validated piezo electric microsensor for repeated measures of IAP. This data demonstrates that femoral pressures are a valid, less invasive method for determining IAP in a standing horse over a range of IAP.

In the final study, serum biomarkers including IL-10, IFABP, and PCT were not correlated or influenced by a period of elevated IAP in healthy, standing horses. The levels of all three biomarkers did not differ over time. However, all three biomarkers were present in serum at the baseline timepoint. While PCT has been reported in the serum of healthy horses, IL-10 and IFABP have not been observed to the author's knowledge. Without a control group, and further investigations into the accuracy of the ELISA used, this data cannot be further analyzed.

The results of the first study are consistent with the laws of hemodynamics of venous return to the heart, which are affected by IAP, inferior vena cava compression, gravity, and right atrial pressure. This study provides validation of the first indirect method for IAP measurement in the horse. Further studies are warranted to evaluate FVP in horses as risk for IAH. Investigation into the use of serum biomarkers does not support the use of a commercial ELISA for analyzing the effects of IAH in horses, without additional validation. Biomarker analysis in the horse for diagnostic and predictive purposes will require additional development and authentication of ELISA tests specific for this species.

References

1.   Malbrain ML, Chiumello D, Pelosi P, et al. Incidence and prognosis of intraabdominal hypertension in a mixed population of critically ill patients: a multiple-center epidemiological study. *Crit Care Med* 2005;33:315-322.

2.   Malbrain ML, Chiumello D, Pelosi P, et al. Prevalence of intra-abdominal hypertension in critically ill patients: a multicentre epidemiological study. *Intensive Care Med* 2004;30:822-829.

3.   Biffl WL, Moore EE, Burch JM, et al. Secondary abdominal compartment syndrome is a highly lethal event. *Am J Surg* 2001;182:645-648.

4.   Greenhalgh DG, Warden GD. The importance of intra-abdominal pressure measurements in burned children. *J Trauma* 1994;36:685-690.

5.   Hobson KG, Young KM, Ciraulo A, et al. Release of abdominal compartment syndrome improves survival in patients with burn injury. *J Trauma* 2002;53:1129-1133; discussion 1133-1124.

6.   Ivy ME, Atweh NA, Palmer J, et al. Intra-abdominal hypertension and abdominal compartment syndrome in burn patients. *J Trauma* 2000;49:387-391.

7.   Kirkpatrick AW, Roberts DJ, De Waele J, et al. Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. *Intensive Care Med* 2013;39:1190-1206.

8.   Malbrain ML, Cheatham ML, Kirkpatrick A, et al. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. I. Definitions. *Intensive Care Med* 2006;32:1722-1732.

9.   Wilkins PA. Abdominal compartment syndrome in equine medicine. American College of Veterinary Internal

Medicine Congress 2005;192-194.

10.   Brosnahan MM, Holbrook TC, Gilliam LL, et al. Intra-abdominal hypertension in two adult horses. *J Vet Emerg Crit Care (San Antonio)* 2009;19:174-180.

11.   Munsterman AS, Hanson RR. Comparison of direct and indirect methods of intra-abdominal pressure measurement in normal horses. *J Vet Emerg Crit Care (San Antonio)* 2009;19:545-553.

12.   Munsterman AS, Hanson RR. Evaluation of gastric pressures as an indirect method for measurement of intraabdominal pressures in the horse. *J Vet Emerg Crit Care (San Antonio)* 2011;21:29-35.

13.   Canola PA, Perotta JH, Dias DPM, et al. Alternative Intrabladder Manometry Technique for the Indirect Measurement of Intra-abdominal Pressure in Horses. *Journal of Equine Veterinary Science* 2012;32:183-189.

14.   Canola PA, Perotta JH, Laskoski LM, et al. Use of gastric balloon manometry for estimation of intra-abdominal pressure in horses. *Equine Vet J* 2011;43:714-720.

15.   Munsterman AS. Intra-abdominal pressure measurement in the horse. American College of Veterinary Surgeon's Veterinary Symposium 2010.

16.   Barrett EJ, Munsterman AS, Albanese V, et al. Direct Measurement of Intra-abdominal Pressures in a Horse by Using a Solid Microsensor. *Journal of Equine Veterinary Science* 2013;33:1000-1003.

17.   Malbrain ML, Deeren D, De Potter TJ. Intra-abdominal hypertension in the critically ill: it is time to pay attention. *Curr Opin Crit Care* 2005;11:156-171.

18.   Sanchez NC, Tenofsky PL, Dort JM, et al. What is normal intra-abdominal pressure? *Am Surg* 2001;67:243-248.

19.   Schein M, Wittmann DH, Aprahamian CC, et al. The abdominal compartment syndrome: the physiological and clinical consequences of elevated intra-abdominal pressure. *J Am Coll Surg* 1995;180:745-753.

20.   De Keulenaer BL, De Waele JJ, Powell B, et al. What is normal intra-abdominal pressure and how is it affected by positioning, body mass and positive end-expiratory pressure? *Intensive Care Med* 2009;35:969-976.

21.   Carr JA. Abdominal compartment syndrome: a decade of progress. *J Am Coll Surg* 2013;216:135-146.

22.   Kron IL, Harman PK, Nolan SP. The measurement of intra-abdominal pressure as a criterion for abdominal re-exploration. *Ann Surg* 1984;199:28-30.

23.   Sugerman H, Windsor A, Bessos M, et al. Intra-abdominal pressure, sagittal abdominal diameter and obesity comorbidity. *J Intern Med* 1997;241:71-79.

24.   Arfvidsson B, Eklof B, Balfour J. Iliofemoral venous pressure correlates with intraabdominal pressure in morbidly obese patients. *Vasc Endovascular Surg* 2005;39:505-509.

25.   Wagoner G. Studies on intra-abdominal pressure. *Am J Med Sci* 1926;171:697-707.

26.   Overholt RH. Intraperitoneal pressure. *Archives of Surgery* 1931;22:691-703.

27.   Scott VH, Williams JM, Mudge MC, et al. Effect of body position on intra-abdominal pressures and abdominal perfusion pressures measured at three sites in horses anesthetized with short-term total intravenous anesthesia. *Am J Vet Res* 2014;75:301-308.

28.   Decramer M, De Troyer A, Kelly S, et al. Regional differences in abdominal pressure swings in dogs. *J Appl Physiol Respir Environ Exerc Physiol* 1984;57:1682-1687.

29.   Cheatham ML, White MW, Sagraves SG, et al. Abdominal perfusion pressure: a superior parameter in the assessment of intra-abdominal hypertension. *J Trauma* 2000;49:621-626; discussion 626-627.

30.   Bouma GJ, Muizelaar JP. Relationship between cardiac output and cerebral blood flow in patients with intact and with impaired autoregulation. *J Neurosurg* 1990;73:368-374.

31.   Cheatham ML, Malbrain ML. Cardiovascular implications of abdominal compartment syndrome. *Acta Clin Belg* 2007;62 Suppl 1:98-112.

32.   Cheatham ML, Malbrain ML, Kirkpatrick A, et al. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. II. Recommendations. *Intensive Care Med* 2007;33:951-962.

33.   Malbrain ML, Peeters Y, Wise R. The neglected role of abdominal compliance in organ-organ interactions. *Crit Care* 2016;20:67.

34.   Song C, Alijani A, Frank T, et al. Mechanical properties of the human abdominal wall measured in vivo during insufflation for laparoscopic surgery. *Surg Endosc* 2006;20:987-990.

35.   Vlot J, Wijnen R, Stolker RJ, et al. Optimizing working space in laparoscopy: CT measurement of the effect of pre-stretching of the abdominal wall in a porcine model. *Surg Endosc* 2014;28:841-846.

36.   Accarino A, Perez F, Azpiroz F, et al. Abdominal distention results from caudo-ventral redistribution of contents. *Gastroenterology* 2009;136:1544-1551.

37.   Villoria A, Azpiroz F, Soldevilla A, et al. Abdominal accommodation: a coordinated adaptation of the abdominal wall to its content. *Am J Gastroenterol* 2008;103:2807-2815.

38.   Song C, Alijani A, Frank T, et al. Elasticity of the living abdominal wall in laparoscopic surgery. *J Biomech* 2006;39:587-591.

39.   Malbrain ML, De Laet I, De Waele JJ, et al. The role of abdominal compliance, the neglected parameter in critically ill patients - a consensus review of 16. Part 2: measurement techniques and management recommendations. *Anaesthesiol Intensive Ther* 2014;46:406-432.

40.   Malbrain ML, Roberts DJ, De Laet I, et al. The role of abdominal compliance, the neglected parameter in critically ill patients - a consensus review of 16. Part 1: definitions and pathophysiology. *Anaesthesiol Intensive Ther* 2014;46:392-405.

41.   Diebel LN, Dulchavsky SA, Brown WJ. Splanchnic ischemia and bacterial translocation in the abdominal compartment syndrome. *J Trauma* 1997;43:852-855.

42.   Bloomfield GL, Ridings PC, Blocher CR, et al. A proposed relationship between increased intra-abdominal, intrathoracic, and intracranial pressure. *Crit Care Med* 1997;25:496-503.

43.   Quintel M, Pelosi P, Caironi P, et al. An increase of abdominal pressure increases pulmonary edema in oleic acid-induced lung injury. *Am J Respir Crit Care Med* 2004;169:534-541.

44.   Sugrue M, Jones F, Deane SA, et al. Intra-abdominal hypertension is an independent cause of postoperative renal impairment. *Arch Surg* 1999;134:1082-1085.

45.   Fietsam R, Jr., Villalba M, Glover JL, et al. Intra-abdominal compartment syndrome as a complication of ruptured abdominal aortic aneurysm repair. *Am Surg* 1989;55:396-402.

46.   Schein M, Ivatury R. Intra-abdominal hypertension and the abdominal compartment syndrome. *Br J Surg* 1998;85:1027-1028.

47.   Meldrum DR, Moore FA, Moore EE, et al. Prospective characterization and selective management of the abdominal compartment syndrome. *Am J Surg* 1997;174:667-672; discussion 672-663.

48.   Eddy V, Nunn C, Morris JA, Jr. Abdominal compartment syndrome. The Nashville experience. *Surg Clin North Am* 1997;77:801-812.

49.   Stassen NA, Lukan JK, Dixon MS, et al. Abdominal compartment syndrome. *Scand J Surg* 2002;91:104-108.

50.   Malbrain ML, Wilmer A. The polycompartment syndrome: towards an understanding of the interactions between different compartments! *Intensive Care Med* 2007;33:1869-1872.

51.   Malbrain ML, Roberts DJ, Sugrue M, et al. The polycompartment syndrome: a concise state-of-the-art review. *Anaesthesiol Intensive Ther* 2014;46:433-450.

52.   Maxwell RA, Fabian TC, Croce MA, et al. Secondary abdominal compartment syndrome: an underappreciated manifestation of severe hemorrhagic shock. *J Trauma* 1999;47:995-999.

53.   Sharma A, Dahiya D, Kaman L, et al. Effect of various pneumoperitoneum pressures on femoral vein hemodynamics during laparoscopic cholecystectomy. *Updates Surg* 2016;68:163-169.

54.   Balogh Z, McKinley BA, Holcomb JB, et al. Both primary and secondary abdominal compartment syndrome can be predicted early and are harbingers of multiple organ failure. *J Trauma* 2003;54:848-859; discussion 859-861.

55.   De Waele J, Desender L, De Laet I, et al. Abdominal decompression for abdominal compartment syndrome in critically ill patients: a retrospective study. *Acta Clin Belg* 2010;65:399-403.

56.   Ivatury RR, Porter JM, Simon RJ, et al. Intra-abdominal hypertension after life-threatening penetrating abdominal trauma: prophylaxis, incidence, and clinical relevance to gastric mucosal pH and abdominal compartment syndrome. *J Trauma* 1998;44:1016-1021; discussion 1021-1013.

57.   Chen J, Ren J, Zhang W, et al. Open versus closed abdomen treatment on liver function in rats with sepsis and abdominal compartment syndrome. *J Trauma* 2011;71:1319-1325; discussion 1325-1316.

58.   Hack CE, Hart M, van Schijndel RJ, et al. Interleukin-8 in sepsis: relation to shock and inflammatory mediators. *Infect Immun* 1992;60:2835-2842.

59.   Fink MP. Gastrointestinal mucosal injury in experimental models of shock, trauma, and sepsis. *Crit Care Med* 1991;19:627-641.

60.   Regueira T, Bruhn A, Hasbun P, et al. Intra-abdominal hypertension: incidence and association with organ dysfunction during early septic shock. *J Crit Care* 2008;23:461-467.

61.   Shoemaker WC, Appel PL, Kram HB, et al. Prospective trial of supranormal values of survivors as therapeutic goals in high-risk surgical patients. *Chest* 1988;94:1176-1186.

62.   Bishop MH, Shoemaker WC, Appel PL, et al. Relationship between supranormal circulatory values, time delays, and outcome in severely traumatized patients. *Crit Care Med* 1993;21:56-63.

63.   Miller PR, Meredith JW, Chang MC. Randomized, prospective comparison of increased preload versus inotropes in the resuscitation of trauma patients: effects on cardiopulmonary function and visceral perfusion. *J Trauma* 1998;44:107-113.

64.   Rivers E, Nguyen B, Havstad S, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001;345:1368-1377.

65.   Jiang LB, Zhang M, Jiang SY, et al. Early goal-directed resuscitation for patients with severe sepsis and septic shock: a meta-analysis and trial sequential analysis. *Scand J Trauma Resusc Emerg Med* 2016;24:23.

66.   Ridings PC, Bloomfield GL, Blocher CR, et al. Cardiopulmonary effects of raised intra-abdominal pressure before and after intravascular volume expansion. *J Trauma* 1995;39:1071-1075.

67.   Boyd JH, Forbes J, Nakada TA, et al. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. *Crit Care Med* 2011;39:259-265.

68.   Balogh Z, McKinley BA, Cocanour CS, et al. Supranormal trauma resuscitation causes more cases of abdominal compartment syndrome. *Arch Surg* 2003;138:637-642; discussion 642-633.

69.   Balogh Z, McKinley BA, Cocanour CS, et al. Patients with impending abdominal compartment syndrome do not respond to early volume loading. *Am J Surg* 2003;186:602-607; discussion 607-608.

70.   Barnes GE, Laine GA, Giam PY, et al. Cardiovascular responses to elevation of intra-abdominal hydrostatic pressure. *Am J Physiol* 1985;248:R208-213.

71.   Richardson JD, Trinkle JK. Hemodynamic and respiratory alterations with increased intra-abdominal pressure. *J Surg Res* 1976;20:401-404.

72.   Kashtan J, Green JF, Parsons EQ, et al. Hemodynamic effect of increased abdominal pressure. *J Surg Res* 1981;30:249-255.

73.   Caldwell CB, Ricotta JJ. Changes in visceral blood flow with elevated intraabdominal pressure. *J Surg Res* 1987;43:14-20.

74.   Schachtrupp A, Graf J, Tons C, et al. Intravascular volume depletion in a 24-hour porcine model of intra-abdominal hypertension. *J Trauma* 2003;55:734-740.

75.   Ivankovich AD, Miletich DJ, Albrecht RF, et al. Cardiovascular effects of intraperitoneal insufflation with carbon dioxide and nitrous oxide in the dog. *Anesthesiology* 1975;42:281-287.

76.   Cullen DJ, Coyle JP, Teplick R, et al. Cardiovascular, pulmonary, and renal effects of massively increased intra-abdominal pressure in critically ill patients. *Crit Care Med* 1989;17:118-121.

77.   Coombs HC. The mechanism of the regulation of intra-abdominal pressure. *Am J Physiol* 1922;61:159-170.

78.   Katz AM. Ernest Henry Starling, his predecessors, and the "Law of the Heart". *Circulation* 2002;106:2986-2992.

79.   Diamant M, Benumof JL, Saidman LJ. Hemodynamics of increased intra-abdominal pressure: Interaction with hypovolemia and halothane anesthesia. *Anesthesiology* 1978;48:23-27.

80.   Takata M, Wise RA, Robotham JL. Effects of abdominal pressure on venous return: abdominal vascular zone conditions. *J Appl Physiol (1985)* 1990;69:1961-1972.

81.   Cheatham ML, Nelson LD, Chang MC, et al. Right ventricular end-diastolic volume index as a predictor of preload status in patients on positive end-expiratory pressure. *Crit Care Med* 1998;26:1801-1806.

82.   Ameloot K, Gillebert C, Desie N, et al. Hypoperfusion, shock states, and abdominal compartment syndrome (ACS). *Surg Clin North Am* 2012;92:207-220, vii.

83.   Cheatham ML, Safcsak K, Block EF, et al. Preload assessment in patients with an open abdomen. *J Trauma* 1999;46:16-22.

84.   Malbrain M. Abdominal Pressure in the critically ill. *Curr Opin Crit Care* 2000;6:17-29.

85.   Wauters J, Wilmer A, Valenza F. Abdomino-thoracic transmission during ACS: facts and figures. *Acta Clin Belg* 2007;62 Suppl 1:200-205.

86.   Malbrain MLNGDL, Inneke. A new concept: The polycompartment syndrome - Part 1. *International Journal of Intensive Care* 2008;15:92-97.

87.   Malbrain MLNGDL, Inneke. A new concept: The polycompartment syndrome - Part 2. *International Journal of Intensive Care* 2009;16:24-31.

88.   Kelman GR, Swapp GH, Smith I, et al. Caridac output and arterial blood-gas tension during laparoscopy. *Br J Anaesth* 1972;44:1155-1162.

89.   Obeid F, Saba A, Fath J, et al. Increases in intra-abdominal pressure affect pulmonary compliance. *Arch Surg* 1995;130:544-547; discussion 547-548.

90.   Pelosi P, Quintel M, Malbrain ML. Effect of intra-abdominal pressure on respiratory mechanics. *Acta Clin Belg* 2007;62 Suppl 1:78-88.

91.   De Laet I, Citerio G, Malbrain ML. The influence of intra-abdominal hypertension on the central nervous system: current insights and clinical recommendations, is it all in the head? *Acta Clin Belg* 2007;62 Suppl 1:89-97.

92.   Bloomfield GL, Dalton JM, Sugerman HJ, et al. Treatment of increasing intracranial pressure secondary to the acute abdominal compartment syndrome in a patient with combined abdominal and head trauma. *J Trauma* 1995;39:1168-1170.

93.   Harman PK, Kron IL, McLachlan HD, et al. Elevated intra-abdominal pressure and renal function. *Ann Surg* 1982;196:594-597.

94.   Bradley SE, Mudge GH, Blake WD, et al. The effect of increased intra-abdominal pressure on the renal excretion of water and electrolytes in normal human subjects and in patients with diabetes insipidus. *Acta Clin Belg* 1955;10:209-223.

95.   Ulyatt DB. Elevated intra-abdominal pressure. *Australian Anaes* 1992:108-114.

96.   Sugrue M, Buist MD, Hourihan F, et al. Prospective study of intra-abdominal hypertension and renal function after laparotomy. *Br J Surg* 1995;82:235-238.

97.   Platell CF, Hall J, Clarke G, et al. Intra-abdominal pressure and renal function after surgery to the abdominal aorta. *Aust N Z J Surg* 1990;60:213-216.

98.   Baker JW, Deitch EA, Li M, et al. Hemorrhagic shock induces bacterial translocation from the gut. *J Trauma* 1988;28:896-906.

99.   Le Roith D, Bark H, Nyska M, et al. The effect of abdominal pressure on plasma antidiuretic hormone levels in the dog. *J Surg Res* 1982;32:65-69.

100.   Diebel LN, Dulchavsky SA, Wilson RF. Effect of increased intra-abdominal pressure on mesenteric arterial and intestinal mucosal blood flow. *J Trauma* 1992;33:45-48; discussion 48-49.

101.   Gudmundsson FF, Gislason HG, Dicko A, et al. Effects of prolonged increased intra-abdominal pressure on gastrointestinal blood flow in pigs. *Surg Endosc* 2001;15:854-860.

102.   Olofsson PH, Berg S, Ahn HC, et al. Gastrointestinal microcirculation and cardiopulmonary function during experimentally increased intra-abdominal pressure. *Crit Care Med* 2009;37:230-239.

103.   Masey SA, Koehler RC, Ruck JR, et al. Effect of abdominal distension on central and regional hemodynamics in neonatal lambs. *Pediatr Res* 1985;19:1244-1249.

104.   Malbrain ML, De laet IE. Intra-abdominal hypertension: evolving concepts. *Clin Chest Med* 2009;30:45-70, viii.

105.   Diebel LN, Wilson RF, Dulchavsky SA, et al. Effect of increased intra-abdominal pressure on hepatic arterial, portal venous, and hepatic microcirculatory blood flow. *J Trauma* 1992;33:279-282; discussion 282-273.

106.   Wendon J. BG, Auzinger G. Intra-abdominal hypertension and the liver. In: Ivatury R. CM, Malbrain M., et al, ed. *Abdominal compartment syndrome*. Georgetown, TX: Landes Bioscience, 2006;138–143.

107.   Inal MT, Memis D, Sezer YA, et al. Effects of intra-abdominal pressure on liver function assessed with the LiMON in critically ill patients. *Can J Surg* 2011;54:161-166.

108.   Michelet P, Roch A, Gainnier M, et al. Influence of support on intra-abdominal pressure, hepatic kinetics of indocyanine green and extravascular lung water during prone positioning in patients with ARDS: a randomized crossover study. *Crit Care* 2005;9:R251-257.

109.   Dalfino L, Malcangi V, Cinnella G, et al. Abdominal hypertension and liver dysfunction in intensive care unit patients: an "on-off" phenomenon? *Transplant Proc* 2006;38:838-840.

110.   Chaves N, Jr., Magalhaes Lde T, Colleoni R, et al. Effects of increased intra-abdominal pressure on the healing process after surgical stapling of the stomach of dogs. *Acta Cir Bras* 2007;22:379-386.

111.   Emerson H. Intra-abdominal pressures. *Arch Intern Med* 1911;7:754-784.

112.   Rushmer RF. The nature of intraperitoneal and intrarectal pressures. *Am J Physiol* 1946;147:242-249.

113.   Malbrain ML. Different techniques to measure intra-abdominal pressure (IAP): time for a critical re-appraisal. *Intensive Care Med* 2004;30:357-371.

114.   Loring SH, Yoshino K, Kimball WR, et al. Gravitational and shear-associated pressure gradients in the abdomen. *J Appl Physiol (1985)* 1994;77:1375-1382.

115.   Cheatham ML, Safcsak K. Is the evolving management of intra-abdominal hypertension and abdominal compartment syndrome improving survival? *Crit Care Med* 2010;38:402-407.

116.   De Waele JJ, De laet I, Malbrain ML. Rational intraabdominal pressure monitoring: how to do it? *Acta Clin Belg* 2007;62 Suppl 1:16-25.

117.   Balogh Z, De Waele JJ, Malbrain ML. Continuous intra-abdominal pressure monitoring. *Acta Clin Belg* 2007;62 Suppl 1:26-32.

118.   De Waele JJ, Cheatham ML, Malbrain ML, et al. Recommendations for research from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. *Acta Clin Belg* 2009;64:203-209.

119.   Kirkpatrick AW, Brenneman FD, McLean RF, et al. Is clinical examination an accurate indicator of raised intra-abdominal pressure in critically injured patients? *Can J Surg* 2000;43:207-211.

120.   Sugrue M, Bauman A, Jones F, et al. Clinical examination is an inaccurate predictor of intraabdominal pressure. *World J Surg* 2002;26:1428-1431.

121.   Malbrain ML, De laet I, Van Regenmortel N, et al. Can the abdominal perimeter be used as an accurate estimation of intra-abdominal pressure? *Crit Care Med* 2009;37:316-319.

122.   Castren M, Liukko K, Nurmi J, et al. Measurement of the abdominal circumference for the detection of intra-abdominal hemorrhage has no diagnostic value. *Acta Anaesthesiol Scand* 2004;48:592-594.

123.   Iberti TJ, Kelly KM, Gentili DR, et al. A simple technique to accurately determine intra-abdominal pressure. *Crit Care Med* 1987;15:1140-1142.

124.   Iberti TJ, Lieber CE, Benjamin E. Determination of intra-abdominal pressure using a transurethral bladder catheter: clinical validation of the technique. *Anesthesiology* 1989;70:47-50.

125.   Cheatham ML, Safcsak K. Intraabdominal pressure: a revised method for measurement. *J Am Coll Surg* 1998;186:594-595.

126.   Balogh Z, Jones F, D'Amours S, et al. Continuous intra-abdominal pressure measurement technique. *Am J Surg* 2004;188:679-684.

127.   Zengerink I, McBeth PB, Zygun DA, et al. Validation and experience with a simple continuous intra-abdominal pressure measurement technique in a multidisciplinary medical/surgical critical care unit. *J Trauma* 2008;64:1159-1164.

128.   Lee SL, Anderson JT, Kraut EJ, et al. A simplified approach to the diagnosis of elevated intra-abdominal pressure. *J Trauma* 2002;52:1169-1172.

129.   Harrahill M. Intra-abdominal pressure monitoring. *J Emerg Nurs* 1998;24:465-466.

130.   De Potter TJ, Dits H, Malbrain ML. Intra- and interobserver variability during in vitro validation of two novel methods for intra-abdominal pressure monitoring. *Intensive Care Med* 2005;31:747-751.

131.   Fusco MA, Martin RS, Chang MC. Estimation of intra-abdominal pressure by bladder pressure measurement: validity and methodology. *J Trauma* 2001;50:297-302.

132.   Yol S, Kartal A, Tavli S, et al. Is urinary bladder pressure a sensitive indicator of intra-abdominal pressure? *Endoscopy* 1998;30:778-780.

133.   Lacey SR, Bruce J, Brooks SP, et al. The relative merits of various methods of indirect measurement of intraabdominal pressure as a guide to closure of abdominal wall defects. *J Pediatr Surg* 1987;22:1207-1211.

134.   Jakob SM, Knuesel R, Tenhunen JJ, et al. Increasing abdominal pressure with and without PEEP: effects on intra-peritoneal, intra-organ and intra-vascular pressures. *BMC Gastroenterol* 2010;10:70.

135.   Johna S, Taylor E, Brown C, et al. Abdominal compartment syndrome: does intra-cystic pressure reflect actual intra-abdominal pressure? A prospective study in surgical patients. *Crit Care* 1999;3:135-138.

136.   Gudmundsson FF, Viste A, Gislason H, et al. Comparison of different methods for measuring intra-abdominal pressure. *Intensive Care Med* 2002;28:509-514.

137.   De Waele J, Pletinckx P, Blot S, et al. Saline volume in transvesical intra-abdominal pressure measurement: enough is enough. *Intensive Care Med* 2006;32:455-459.

138.   Ball CG, Kirkpatrick AW. 'Progression towards the minimum': the importance of standardizing the priming volume during the indirect measurement of intra-abdominal pressures. *Crit Care* 2006;10:153.

139.   De laet I, Hoste E, De Waele JJ. Transvesical intra-abdominal pressure measurement using minimal instillation volumes: how low can we go? *Intensive Care Med* 2008;34:746-750.

140.   De Keulenaer BL, Cheatham ML, De Waele JJ, et al. Intra-abdominal pressure measurements in lateral decubitus versus supine position. *Acta Clin Belg* 2009;64:210-215.

141. Risin E, Kessel B, Lieberman N, et al. New technique of direct intra-abdominal pressure measurement. *Asian J Surg* 2006;29:247-250.

142. Conzemius MG, Sammarco JL, Holt DE, et al. Clinical determination of preoperative and postoperative intra-abdominal pressures in dogs. *Vet Surg* 1995;24:195-201.

143. Rader RA, Johnson JA. Determination of normal intra-abdominal pressure using urinary bladder catheterization in clinically healthy cats. *J Vet Emerg Crit Care (San Antonio)* 2010;20:386-392.

144. Bosch L, Rivera del Alamo MM, Andaluz A, et al. Effects of ovariohysterectomy on intra-abdominal pressure and abdominal perfusion pressure in cats. *Vet Rec* 2012;171:622.

145. Fetner M, Prittie J. Evaluation of transvesical intra-abdominal pressure measurement in hospitalized dogs. *J Vet Emerg Crit Care (San Antonio)* 2012;22:230-238.

146. Kimball EJ, Mone MC, Wolfe TR, et al. Reproducibility of bladder pressure measurements in critically ill patients. *Intensive Care Med* 2007;33:1195-1198.

147. Way LI, Monnet E. Determination and validation of volume to be instilled for standardized intra-abdominal pressure measurement in dogs. *J Vet Emerg Crit Care (San Antonio)* 2014;24:403-407.

148. Southwood L. L. WPA. Measurement of intra-abdominal pressure in normal horses. 23rd American College of Veterinary Internal Medicine Congress 2005;190-191.

149. Parry BW, McCarthy MA, Anderson GA. Survey of resting blood pressure values in clinically normal horses. *Equine Vet J* 1984;16:53-58.

150. van Waes OJ, Jaquet JB, Hop WC, et al. A Single-Lumen Central Venous Catheter for Continuous and Direct Intra-abdominal Pressure Measurement. *Eur J Trauma Emerg Surg* 2009;35:532-537.

151. Davis PJ, Koottayi S, Taylor A, et al. Comparison of indirect methods of measuring intra-abdominal pressure in children. *Intensive Care Med* 2005;31:471-475.

152. Suominen PK, Pakarinen MP, Rautiainen P, et al. Comparison of direct and intravesical measurement of intraabdominal pressure in children. *J Pediatr Surg* 2006;41:1381-1385.

153.   Correa-Martin L, Castellanos G, Garcia-Lindo M, et al. [Intra-abdominal hypertension: effects on the splanchnic circulation. Preliminary study in a model of ascites]. *Gastroenterol Hepatol* 2014;37:51-57.

154.   Risin E, Kessel B, Ashkenazi I, et al. A new technique of direct intra-abdominal pressure measurement: a preliminary study. *Am J Surg* 2006;191:235-237.

155.   Schachtrupp A, Tons C, Fackeldey V, et al. Evaluation of two novel methods for the direct and continuous measurement of the intra-abdominal pressure in a porcine model. *Intensive Care Med* 2003;29:1605-1608.

156.   Schachtrupp A, Henzler D, Orfao S, et al. Evaluation of a modified piezoresistive technique and a water-capsule technique for direct and continuous measurement of intra-abdominal pressure in a porcine model. *Crit Care Med* 2006;34:745-750.

157.   Brooks AJ, Simpson A, Delbridge M, et al. Validation of direct intraabdominal pressure measurement using a continuous indwelling compartment pressure monitor. *J Trauma* 2005;58:830-832.

158.   Hurcombe SD, Scott VH. Direct intra-abdominal pressures and abdominal perfusion pressures in unsedated normal horses. *J Vet Emerg Crit Care (San Antonio)* 2012;22:441-446.

159.   Dechant D. E. N, J. E., LeJeune S. S. Comparison of techniques for measurement of intra-abdominal pressure in horses. 10th American College of Veterinary Emergency and Critical Care Symposium 2008;838.

160.   Collee GG, Lomax DM, Ferguson C, et al. Bedside measurement of intra-abdominal pressure (IAP) via an indwelling naso-gastric tube: clinical validation of the technique. *Intensive Care Med* 1993;19:478-480.

161.   Campbell EJ, Green JH. The variations in intra-abdominal pressure and the activity of the abdominal muscles during breathing; a study in man. *J Physiol* 1953;122:282-290.

162.   Mills JN. The pressures developed in abdomen and thorax during the Flack tests. *J Physiol* 1950;111:368-375.

163.   Sugrue M, Buist MD, Lee A, et al. Intra-abdominal pressure measurement using a modified nasogastric tube: description and validation of a new technique. *Intensive Care Med* 1994;20:588-590.

164.   Engum SA, Kogon B, Jensen E, et al. Gastric tonometry and direct intraabdominal pressure monitoring in abdominal compartment syndrome. *J Pediatr Surg* 2002;37:214-218.

165. Turnbull D, Webber S, Hamnegard CH, et al. Intra-abdominal pressure measurement: validation of intragastric pressure as a measure of intra-abdominal pressure. *Br J Anaesth* 2007;98:628-634.

166. De Waele JJ, Billiet EA, Hoste E, et al. Fluid vs. air for semicontinuous intra-abdominal pressure measurements using a compliance catheter. *Intensive Care Med* 2005;31:598-599.

167. De Waele JJ, Berrevoet F, Reyntjens K, et al. Semicontinuous intra-abdominal pressure measurement using an intragastric Compliance catheter. *Intensive Care Med* 2007;33:1297-1300.

168. Moore FA, Cocanour CS, McKinley BA, et al. Migrating motility complexes persist after severe traumatic shock in patients who tolerate enteral nutrition. *J Trauma* 2001;51:1075-1082.

169. Slocumbe RB, K.; Covell, G.; Bayly, W. Effect of treadmill exercise on intrapleural, transdiaphragmatic and intra-abdominal pressures in Standardbred horses. *Equine Exercise Physiology* 1991;3:83-91.

170. Art T, Anderson L, Woakes AJ, et al. Mechanics of breathing during strenuous exercise in Thoroughbred horses. *Respir Physiol* 1990;82:279-294.

171. Ainsworth DM, Eicker SW, Nalevanko ME, et al. The effect of exercise on diaphragmatic activation in horses. *Respir Physiol* 1996;106:35-46.

172. Koterba AM, Kosch PC, Beech J, et al. Breathing strategy of the adult horse (Equus caballus) at rest. *J Appl Physiol (1985)* 1988;64:337-346.

173. Williams JF, Seneff MG, Friedman BC, et al. Use of femoral venous catheters in critically ill adults: prospective study. *Crit Care Med* 1991;19:550-553.

174. Kanter RK, Zimmerman JJ, Strauss RH, et al. Central venous catheter insertion by femoral vein: safety and effectiveness for the pediatric patient. *Pediatrics* 1986;77:842-847.

175. Stenzel JP, Green TP, Fuhrman BP, et al. Percutaneous central venous catheterization in a pediatric intensive care unit: a survival analysis of complications. *Crit Care Med* 1989;17:984-988.

176. Yung M, Butt W. Inferior vena cava pressure as an estimate of central venous pressure. *J Paediatr Child Health* 1995;31:399-402.

177. Getzen LC, Pollak EW. Short-term femoral vein catheterization. A safe alternative venous access? *Am J Surg* 1979;138:875-878.

178.   Thomas F, Burke JP, Parker J, et al. The risk of infection related to radial vs femoral sites for arterial catheterization. *Crit Care Med* 1983;11:807-812.

179.   Murr MM, Rosenquist MD, Lewis RW, 2nd, et al. A prospective safety study of femoral vein versus nonfemoral vein catheterization in patients with burns. *J Burn Care Rehabil* 1991;12:576-578.

180.   Markwalder J, Starling EH. On the constancy of the systolic output under varying conditions. *J Physiol* 1914;48:348-356.

181.   Patterson SW, Starling EH. On the mechanical factors which determine the output of the ventricles. *J Physiol* 1914;48:357-379.

182.   Knowlton FP, Starling EH. The influence of variations in temperature and blood-pressure on the performance of the isolated mammalian heart. *J Physiol* 1912;44:206-219.

183.   Regli A, De Keulenaer BL, Hockings LE, et al. The role of femoral venous pressure and femoral venous oxygen saturation in the setting of intra-abdominal hypertension: a pig model. *Shock* 2011;35:422-427.

184.   Ishizaki Y, Bandai Y, Shimomura K, et al. Safe intraabdominal pressure of carbon dioxide pneumoperitoneum during laparoscopic surgery. *Surgery* 1993;114:549-554.

185.   Duke T, Steinacher SL, Remedios AM. Cardiopulmonary effects of using carbon dioxide for laparoscopic surgery in dogs. *Vet Surg* 1996;25:77-82.

186.   Smith I, Benzie RJ, Gordon NL, et al. Cardiovascular effects of peritoneal insufflation of carbon dioxide for laparoscopy. *Br Med J* 1971;3:410-411.

187.   Markou N, Grigorakos L, Myrianthefs P, et al. Venous pressure measurements in the superior and inferior vena cava: the influence of intra-abdominal pressure. *Hepatogastroenterology* 2004;51:51-55.

188.   Sood P, Dass B, Bakuzonis C, et al. Intra-abdominal hypertension can be monitored with femoral vein catheters during CRRT and may cause access recirculation. *Clin Nephrol* 2010;74:223-228.

189.   De Keulenaer BL, Regli A, Dabrowski W, et al. Does femoral venous pressure measurement correlate well with intrabladder pressure measurement? A multicenter observational trial. *Intensive Care Med* 2011;37:1620-1627.

190.   Howard AE, Regli A, Litton E, et al. Can femoral venous pressure be used as an estimate for standard vesical intra-abdominal pressure measurement? *Anaesth Intensive Care* 2016;44:704-711.

191.   Forstemann T, Trzewik J, Holste J, et al. Forces and deformations of the abdominal wall--a mechanical and geometrical approach to the linea alba. *J Biomech* 2011;44:600-606.

192.   Mulier J, Coenegrachts K, Van De Moortele K. CT analysis of the elastic deformation and elongation of the abdominal wall during colon inflation for virtual coloscopy: 3AP6-7. *European Journal of Anaesthesiology (EJA)* 2008;25:42.

193.   van Ramshorst GH, Salih M, Hop WC, et al. Noninvasive assessment of intra-abdominal pressure by measurement of abdominal wall tension. *J Surg Res* 2011;171:240-244.

194.   Blaser AR, Bjorck M, De Keulenaer B, et al. Abdominal compliance: A bench-to-bedside review. *J Trauma Acute Care Surg* 2015;78:1044-1053.

195.   Sturini E, Saporito A, Sugrue M, et al. Respiratory variation of intra-abdominal pressure: indirect indicator of abdominal compliance? *Intensive Care Med* 2008;34:1632-1637.

196.   Drellich S. Intraabdominal pressure In: Silverstein DCH, K., ed. *Small Animal Critical Care Medicine*. St. Louis: Saunders, Elsevier Inc., 2009;872-874.

197.   Pelosi P, Aspesi M, Gamberoni C, et al. Measuring intra-abdominal pressure in the intensive care setting. *Intensivmedizin und Notfallmedizin* 2002;39:509-519.

198.   Ivatury RR, Diebel L, Porter JM, et al. Intra-abdominal hypertension and the abdominal compartment syndrome. *Surg Clin North Am* 1997;77:783-800.

199.   Malbrain ML, Vidts W, Ravyts M, et al. Acute intestinal distress syndrome: the importance of intra-abdominal pressure. *Minerva Anestesiol* 2008;74:657-673.

200.   Joubert KE, Oglesby PA, Downie J, et al. Abdominal compartment syndrome in a dog with babesiosis. *Journal of Veterinary Emergency and Critical Care* 2007;17:184-190.

201.   Wilkins PA. Abdominal compartment syndrome in equine medicine. 23rd American College of Veterinary Internal Medicine Congress 2005;192-194.

202.   Chiumello D, Tallarini F, Chierichetti M, et al. The effect of different volumes and temperatures of saline on the bladder pressure measurement in critically ill patients. *Crit Care* 2007;11:R82.

203.   Malbrain ML, Deeren DH. Effect of bladder volume on measured intravesical pressure: a prospective cohort study. *Crit Care* 2006;10:R98.

204. Bumaschny ER, A. Abdominal compartment syndrome In: Baue AEF, E.; Fry, D.E., ed. *Multi-organ failure: pathophysiology,*

prevention and therapy. New York: Springer-Verlag, 2000;291-302.

205. Lambert DM, Marceau S, Forse RA. Intra-abdominal pressure in the morbidly obese. *Obes Surg* 2005;15:1225-1232.

206. Malbrain M. Relationship of body mass index (BMI), lactate and intra-abdominal pressure (IAP) to subsequent mortality in ICU patients. *Critical Care* 1999;3:P039-P039.

207. Juozaitiene VS, A.; Augonyte, G. . Horse body mass assessment according to its dimensions. *Veterinarija Ir Zootechnika* 1997;4:1392-1396.

208. Parry BW, Gay CC, McCarthy MA. Influence of head height on arterial blood pressure in standing horses. *Am J Vet Res* 1980;41:1626-1631.

209. Lerner SM. Review article: the abdominal compartment syndrome. *Aliment Pharmacol Ther* 2008;28:377-384.

210. An G, West MA. Abdominal compartment syndrome: a concise clinical review. *Crit Care Med* 2008;36:1304-1310.

211. Shear W, Rosner MH. Acute kidney dysfunction secondary to the abdominal compartment syndrome. *J Nephrol* 2006;19:556-565.

212. Doty JM, Saggi BH, Sugerman HJ, et al. Effect of increased renal venous pressure on renal function. *J Trauma* 1999;47:1000-1003.

213. Lattuada M, Hedenstierna G. Abdominal lymph flow in an endotoxin sepsis model: influence of spontaneous breathing and mechanical ventilation. *Crit Care Med* 2006;34:2792-2798.

214. Malbrain ML, Pelosi P, De laet I, et al. Lymphatic drainage between thorax and abdomen: please take good care of this well-performing machinery. *Acta Clin Belg* 2007;62 Suppl 1:152-161.

215. Cresswell AB, Wendon JA. Hepatic function and non-invasive hepatosplanchnic monitoring in patients with abdominal hypertension. *Acta Clin Belg* 2007;62 Suppl 1:113-118.

216. Sakka SG. Indocyanine green plasma disappearance rate as an indicator of hepato-splanchnic ischemia during abdominal compartment syndrome. *Anesth Analg* 2007;104:1003-1004.

217.   Perkins JD. Abdominal compartment syndrome: is the liver involved? *Liver Transpl* 2006;12:1555-1556.

218.   Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307-310.

219.   Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 1989;45:255-268.

220.   Lin LI. Erratum: A note on the concordance correlation coefficient. *Biometrics* 2000;56:324-325.

221.   Saggi BH, Sugerman HJ, Ivatury RR, et al. Abdominal compartment syndrome. *J Trauma* 1998;45:597-609.

222.   King JN, Gerring EL. The action of low dose endotoxin on equine bowel motility. *Equine Vet J* 1991;23:11-17.

223.   Cruz AM, Kerr CL, Boure LP, et al. Cardiovascular effects of insufflation of the abdomen with carbon dioxide in standing horses sedated with detomidine. *Am J Vet Res* 2004;65:357-362.

224.   Doherty TJ, Andrews FM, Provenza MK, et al. The effect of sedation on gastric emptying of a liquid marker in ponies. *Vet Surg* 1999;28:375-379.

225.   Sanchez LC, Giguere S, Javsicas LH, et al. Effect of age, feeding, and omeprazole administration on gastric tonometry in healthy neonatal foals. *J Vet Intern Med* 2008;22:406-410.

226.   Valverde A, Giguere S, Sanchez LC, et al. Effects of dobutamine, norepinephrine, and vasopressin on cardiovascular function in anesthetized neonatal foals with induced hypotension. *Am J Vet Res* 2006;67:1730-1737.

227.   Tonometric Catheters.

228.   Lorenzo-Figueras M, Merritt AM. Effects of exercise on gastric volume and pH in the proximal portion of the stomach of horses. *Am J Vet Res* 2002;63:1481-1487.

229.   De Laet IE, Ravyts M, Vidts W, et al. Current insights in intra-abdominal hypertension and abdominal compartment syndrome: open the abdomen and keep it open! *Langenbecks Arch Surg* 2008;393:833-847.

230.   Johnson JM, Chang PK, Gagliardi RJ, et al. Abdominal compartment syndrome. *J Surg Educ* 2007;64:208-211.

231. Hunter JD, Damani Z. Intra-abdominal hypertension and the abdominal compartment syndrome. *Anaesthesia* 2004;59:899-907.

232. Pracca FF, Biestro AA, Moraes L, et al. Direct measurement of intra-abdominal pressure with a solid microtranducer. *J Clin Monit Comput* 2007;21:167-170.

233. Barrett EJ, Munsterman AS, Hanson RR. Effects of gastric distension on intraabdominal pressures in horses. *J Vet Emerg Crit Care (San Antonio)* 2013;23:423-428.

234. Magder S. Central venous pressure: A useful but not so simple measurement. *Crit Care Med* 2006;34:2224-2227.

235. Boldt J, Lenz M, Kumle B, et al. Volume replacement strategies on intensive care units: results from a postal survey. *Intensive Care Med* 1998;24:147-151.

236. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. *Crit Care Med* 2013;41:580-637.

237. Lichtwarck-Aschoff M, Zeravik J, Pfeiffer UJ. Intrathoracic blood volume accurately reflects circulatory volume status in critically ill patients with mechanical ventilation. *Intensive Care Med* 1992;18:142-147.

238. Lichtwarck-Aschoff M, Beale R, Pfeiffer UJ. Central venous pressure, pulmonary artery occlusion pressure, intrathoracic blood volume, and right ventricular end-diastolic volume as indicators of cardiac preload. *J Crit Care* 1996;11:180-188.

239. Aliverti A, Bovio D, Fullin I, et al. The abdominal circulatory pump. *PLoS One* 2009;4:e5550.

240. Kitano Y, Takata M, Sasaki N, et al. Influence of increased abdominal pressure on steady-state cardiac performance. *J Appl Physiol (1985)* 1999;86:1651-1656.

241. Keegan RD, Greene SA, Brown JA, et al. Effects of pre-exercise frusemide administration and post exercise anaesthesia on cardiopulmonary and acid-base parameters and blood and plasma volumes in horses exercised supramaximally to fatigue. *Equine Vet J Suppl* 1999:174-177.

242. de Laforcade AM, Rozanski EA. Central venous pressure and arterial blood pressure measurements. *Vet Clin North Am Small Anim Pract* 2001;31:1163-1174, vi.

243. Magdesian KG, Fielding CL, Rhodes DM, et al. Changes in central venous pressure and blood lactate concentration in response to acute blood loss in horses. *J Am Vet Med Assoc* 2006;229:1458-1462.

244.   Aldrich JH, S. C. Monitoring the critically ill patient In: Bonagura JD, ed. *Kirk's Current Veterinary Therapy XII*. Philadelphia, PA: W. B. Saunders, 1995;98-105.

245.   Hansen B. Technical aspects of fluid therapy In: Dibartola SP, ed. *Fluid, electrolyte, and acid base disorders in small animal practice*. Philadelphia, PA: W. B. Saunders, 2006;371-376.

246.   Machon RG, D.V.M MRR, Robinson EP. Central Venous Pressure Measurements in the Caudal Vena Cava of Sedated Cats. *Journal of Veterinary Emergency and Critical Care* 1995;5:121-129.

247.   Oakley RE, Olivier B, Eyster GE, et al. Experimental evaluation of central venous pressure monitoring in the dog. *J Am Anim Hosp Assoc* 1997;33:77-82.

248.   Magder S. Right Atrial Pressure in the Critically Ill: how to measure, what is the value, what are the limitations. *Chest* 2016.

249.   Magder S. Central venous pressure monitoring. *Curr Opin Crit Care* 2006;12:219-227.

250.   Daniels SR, Hannon DW, Meyer RA, et al. Paroxysmal supraventricular tachycardia. A complication of jugular central venous catheters in neonates. *Am J Dis Child* 1984;138:474-475.

251.   Kayashima K. Factors affecting survival in pediatric cardiac tamponade caused by central venous catheters. *Journal of Anesthesia* 2015;29:944-952.

252.   Agarwal KC, Khan MA, Falla A, et al. Cardiac perforation from central venous catheters: survival after cardiac tamponade in an infant. *Pediatrics* 1984;73:333-338.

253.   Lloyd TR, Donnerstein RL, Berg RA. Accuracy of central venous pressure measurement from the abdominal inferior vena cava. *Pediatrics* 1992;89:506-508.

254.   Joynt GM, Gomersall CD, Buckley TA, et al. Comparison of intrathoracic and intra-abdominal measurements of central venous pressure. *Lancet* 1996;347:1155-1157.

255.   McGee SR. Physical examination of venous pressure: a critical review. *Am Heart J* 1998;136:10-18.

256.   Winsor T, Burch GE. Use of the phlebomanometer; normal venous pressure values and a study of certain clinical aspects of venous hypertension in man. *Am Heart J* 1946;31:387-406.

257.   Holt JP. The measurement of venous pressure in man, eliminating the hydrostatic factor. *American Heart Journal* 1940;20:642-643.

258.   Winsor T, Burch GE. Phlebostatic Axis and Phlebostatic Level, Reference Levels for Venous Pressure Measurements in Man. *Proceedings of the Society for Experimental Biology and Medicine* 1945;58:165-169.

259.   Beigel R, Cercek B, Luo H, et al. Noninvasive evaluation of right atrial pressure. *J Am Soc Echocardiogr* 2013;26:1033-1042.

260.   Walsh JT, Hildick-Smith DJ, Newell SA, et al. Comparison of central venous and inferior vena caval pressures. *Am J Cardiol* 2000;85:518-520, A511.

261.   Guyton AC, Greganti FP. A physiologic reference point for measuring circulatory pressures in the dog; particularly venous pressure. *Am J Physiol* 1956;185:137-141.

262.   Reems MM, Aumann M. Central venous pressure: principles, measurement, and interpretation. *Compend Contin Educ Vet* 2012;34:E1.

263.   Russell WJ. *Central Venous Pressure: Its Clinical Use and Role in Cardiovascular Dynamics*: Elsevier Science, 2013.

264.   Norton JL, Nolen-Walston RD, Underwood C, et al. Comparison of water manometry to 2 commercial electronic pressure monitors for central venous pressure measurement in horses. *J Vet Intern Med* 2011;25:303-306.

265.   Wilsterman S, Hackett ES, Rao S, et al. A technique for central venous pressure measurement in normal horses. *J Vet Emerg Crit Care (San Antonio)* 2009;19:241-246.

266.   Klein L, Sherman J. Effects of preanesthetic medication, anesthesia, and position of recumbency on central venous pressure in horses. *J Am Vet Med Assoc* 1977;170:216-219.

267.   Tam K, Rezende M, Boscan P. Correlation between jugular and central venous pressures in laterally recumbent horses. *Vet Anaesth Analg* 2011;38:580-583.

268.   Hall LW, Trim CM. Positive end-expiratory pressure in anaesthetized spontaneously breathing horses. *Br J Anaesth* 1975;47:819-824.

269.   Fielding CL, Balaam JL, K.A. S. How to measure central venous pressure in standing adult horses. American Association of Equine Practitioners 2004;415-417.

270.	Norton JL, Nolen-Walston RD, Underwood C, et al. Repeatability, reproducibility, and effect of head position on central venous pressure measurement in standing adult horses. *J Vet Intern Med* 2011;25:575-578.

271.	Rizvi K, Deboisblanc BP, Truwit JD, et al. Effect of airway pressure display on interobserver agreement in the assessment of vascular pressures in patients with acute lung injury and acute respiratory distress syndrome. *Crit Care Med* 2005;33:98-103; discussion 243-104.

272.	Hall LW, Nigam JM. Papers and articles measurement of central venous pressure in horses. *Vet Rec* 1975;97:66-69.

273.	Klide AM, Calderwood HW, Soma LR. Cardiopulmonary effects of xylazine in dogs. *Am J Vet Res* 1975;36:931-935.

274.	Schmitt H, Fournadjiev G, Schmitt H. Central and peripheral effects of 2-(2,6-dimethylphenylamino)-4-H-5,6-dihydro-1,3-thiazin (Bayer 1470) on the sympathetic system. *Eur J Pharmacol* 1970;10:230-238.

275.	McCashin FB, Gabel AA. Evaluation of xylazine as a sedative and preanesthetic agent in horses. *Am J Vet Res* 1975;36:1421-1429.

276.	Rutkowski JA, Eades SC, Moore JN. Effects of xylazine butorphanol on cecal arterial blood flow, cecal mechanical activity, and systemic hemodynamics in horses. *Am J Vet Res* 1991;52:1153-1158.

277.	Magder S. Understanding central venous pressure: not a preload index? *Curr Opin Crit Care* 2015;21:369-375.

278.	Vinayak AG, Levitt J, Gehlbach B, et al. Usefulness of the external jugular vein examination in detecting abnormal central venous pressure in critically ill patients. *Arch Intern Med* 2006;166:2132-2137.

279.	Schertel ER, Allen DA, Muir WW, et al. Evaluation of a hypertonic saline-dextran solution for treatment of dogs with shock induced by gastric dilatation-volvulus. *J Am Vet Med Assoc* 1997;210:226-230.

280.	Nolen-Walston RD, Norton JL, Navas de Solis C, et al. The effects of hypohydration on central venous pressure and splenic volume in adult horses. *J Vet Intern Med* 2011;25:570-574.

281.	Komori M, Takada K, Tomizawa Y, et al. Effects of colloid resuscitation on peripheral microcirculation, hemodynamics, and colloidal osmotic pressure during acute severe hemorrhage in rabbits. *Shock* 2005;23:377-382.

282.   Kay HR, Afshari M, Barash P, et al. Measurement of ejection fraction by thermal dilution techniques. *J Surg Res* 1983;34:337-346.

283.   Hardy JD, Garcia JB, Hardy JA, et al. Fluid replacement monitoring: effect of dextran overload, norepinephrine drip, and positive pressure ventilation on systemic arterial, right atrial pulmonary wedge, and left atrial pressures in dogs. *Ann Surg* 1974;180:162-166.

284.   Pantaleon LG, Furr MO, McKenzie HC, 2nd, et al. Cardiovascular and pulmonary effects of hetastarch plus hypertonic saline solutions during experimental endotoxemia in anesthetized horses. *J Vet Intern Med* 2006;20:1422-1428.

285.   Marik PE, Baram M, Vahid B. Does central venous pressure predict fluid responsiveness? A systematic review of the literature and the tale of seven mares. *Chest* 2008;134:172-178.

286.   Hollenberg SM, Ahrens TS, Annane D, et al. Practice parameters for hemodynamic support of sepsis in adult patients: 2004 update. *Crit Care Med* 2004;32:1928-1948.

287.   Sasai T, Tokioka H, Fukushima T, et al. Reliability of central venous pressure to assess left ventricular preload for fluid resuscitation in patients with septic shock. *J Intensive Care* 2014;2:58.

288.   Kumar A, Anel R, Bunnell E, et al. Pulmonary artery occlusion pressure and central venous pressure fail to predict ventricular filling volume, cardiac performance, or the response to volume infusion in normal subjects. *Crit Care Med* 2004;32:691-699.

289.   Starling EH. The Linacre lecture of the law of the heart 1918.

290.   Lee EJ, Nedrud J, Schemmel P, et al. Calcium sensitivity and myofilament lattice structure in titin N2B KO mice. *Arch Biochem Biophys* 2013;535:76-83.

291.   Allen DG, Kentish JC. The cellular basis of the length-tension relation in cardiac muscle. *J Mol Cell Cardiol* 1985;17:821-840.

292.   Diebel LN, Wilson RF, Tagett MG, et al. End-diastolic volume. A better indicator of preload in the critically ill. *Arch Surg* 1992;127:817-821; discussion 821-812.

293.   Johnson HD. Critical Closing Pressure in Blood Vessels. *Nature* 1967;215:858-858.

294.   Desmond J, Gordon RA. Ventilation in patients anaesthetized for laparoscopy. *Can Anaesth Soc J* 1970;17:378-387.

295.   Guyton AC, Adkins LH. Quantitative aspects of the collapse factor in relation to venous return. *Am J Physiol* 1954;177:523-527.

296.   Smith HJ, Grottum P, Simonsen S. Ultrasonic assessment of abdominal venous return. II. Volume blood flow in the inferior vena cava and portal vein. *Acta Radiol Diagn (Stockh)* 1986;27:23-27.

297.   Eckstein RW, Wiggers CJ, Graham GR. PHASIC CHANGES IN INFERIOR CAVA FLOW OF INTRAVASCULAR ORIGIN. *American Journal of Physiology -- Legacy Content* 1947;148:740-744.

298.   Mitzner W, Goldberg H. Effects of epinephrine on resisitive and compliant properties of the canine vasculature. *J Appl Physiol* 1975;39:272-280.

299.   Beyar R, Halperin HR, Tsitlik JE, et al. Circulatory assistance by intrathoracic pressure variations: optimization and mechanisms studied by a mathematical model in relation to experimental data. *Circ Res* 1989;64:703-720.

300.   Morgan BC, Abel FL, Mullins GL, et al. Flow patterns in cavae, pulmonary artery, pulmonary vein, and aorta in intact dogs. *Am J Physiol* 1966;210:903-909.

301.   West JB, Dollery CT, Naimark A. DISTRIBUTION OF BLOOD FLOW IN ISOLATED LUNG; RELATION TO VASCULAR AND ALVEOLAR PRESSURES. *J Appl Physiol* 1964;19:713-724.

302.   Brower R, Wise RA, Hassapoyannes C, et al. Effect of lung inflation on lung blood volume and pulmonary venous flow. *J Appl Physiol (1985)* 1985;58:954-963.

303.   Permutt S, Riley RL. Hemodynamics of collapsible vessels with tone: the vascular waterfall. *J Appl Physiol* 1963;18:924-932.

304.   Robotham JL, Wise RA, Bromberger-Barnea B. Effects of changes in abdominal pressure on left ventricular performance and regional blood flow. *Crit Care Med* 1985;13:803-809.

305.   Guyton AC, Lindsey AW, Abernathy B, et al. Venous return at various right atrial pressures and the normal venous return curve. *Am J Physiol* 1957;189:609-615.

306.   Ranniger K, Switz DM. Local Obstruction of the Inferior Vena Cava by Massive Ascites. *Am J Roentgenol Radium Ther Nucl Med* 1965;93:935-939.

307.   Doppman J, Rubinson RM, Rockoff SD, et al. Mechanism of obstruction of the infradiaphragmatic portion of the inferior vena cava in the presence of increased intra-abdominal pressure. *Invest Radiol* 1966;1:37-53.

308.	Rubinson RM, Vasko JS, Doppman JL, et al. Inferior vena caval obstruction from increased intra-abdominal pressure. Experimental hemodynamic and angiographic observations. *Arch Surg* 1967;94:766-770.

309.	Wachsberg RH. Narrowing of the upper abdominal inferior vena cava in patients with elevated intraabdominal pressure: sonographic observations. *J Ultrasound Med* 2000;19:217-222.

310.	Smith HJ, Grottum P, Simonsen S. Ultrasonic assessment of abdominal venous return. I. Effect of cardiac action and respiration on mean velocity pattern, cross-sectional area and flow in the inferior vena cava and portal vein. *Acta Radiol Diagn (Stockh)* 1985;26:581-588.

311.	Lloyd TC, Jr. Effect of inspiration on inferior vena caval blood flow in dogs. *J Appl Physiol Respir Environ Exerc Physiol* 1983;55:1701-1708.

312.	Parmeggiani D, Apperti M, De Pascale V, et al. Abdominal hypertension and venous insufficiency of lower limbs: is there a relationship? *Ann Ital Chir* 2013;84:67-72.

313.	Yang C, Yang Z, Chen X, et al. Inverted U-Shaped Relationship between Central Venous Pressure and Intra-Abdominal Pressure in the Early Phase of Severe Acute Pancreatitis: A Retrospective Study. *PLoS One* 2015;10:e0128493.

314.	Kuntscher MV, Germann G, Hartmann B. Correlations between cardiac output, stroke volume, central venous pressure, intra-abdominal pressure and total circulating blood volume in resuscitation of major burns. *Resuscitation* 2006;70:37-43.

315.	Holm C, Melcer B, Horbrand F, et al. Haemodynamic and oxygen transport responses in survivors and non-survivors following thermal injury. *Burns* 2000;26:25-33.

316.	Schiller WR, Bay RC. Hemodynamic and oxygen transport monitoring in management of burns. *New Horiz* 1996;4:475-482.

317.	Dries DJ, Waxman K. Adequate resuscitation of burn patients may not be measured by urine output and vital signs. *Crit Care Med* 1991;19:327-329.

318.	Kuntscher MV, Blome-Eberwein S, Pelzer M, et al. Transcardiopulmonary vs pulmonary arterial thermodilution methods for hemodynamic monitoring of burned patients. *J Burn Care Rehabil* 2002;23:21-26.

319.	Bodson L, Vieillard-Baron A. Respiratory variation in inferior vena cava diameter: surrogate of central venous pressure or parameter of fluid responsiveness? Let the physiology reply. *Crit Care* 2012;16:181.

320.   Berg RA, Lloyd TR, Donnerstein RL. Accuracy of central venous pressure monitoring in the intraabdominal inferior vena cava: a canine study. *J Pediatr* 1992;120:67-71.

321.   Chait HI, Kuhn MA, Baum VC. Inferior vena caval pressure reliably predicts right atrial pressure in pediatric cardiac surgical patients. *Crit Care Med* 1994;22:219-224.

322.   Nahum E, Dagan O, Sulkes J, et al. A comparison between continuous central venous pressure measurement from right atrium and abdominal vena cava or common iliac vein. *Intensive Care Med* 1996;22:571-574.

323.   Groombridge CJ, Duplooy D, Adams BD, et al. Comparison of central venous pressure and venous oxygen saturation from venous catheters placed in the superior vena cava or via a femoral vein: the numbers are not interchangeable. *Crit Care Resusc* 2011;13:151-155.

324.   Natori H, Tamaki S, Kira S. Ultrasonographic evaluation of ventilatory effect on inferior vena caval configuration. *Am Rev Respir Dis* 1979;120:421-427.

325.   Sobolewski AP, Deshmukh RM, Brunson BL, et al. Venous hemodynamic changes during laparoscopic cholecystectomy. *J Laparoendosc Surg* 1995;5:363-369.

326.   Beebe DS, McNevin MP, Crain JM, et al. Evidence of venous stasis after abdominal insufflation for laparoscopic cholecystectomy. *Surg Gynecol Obstet* 1993;176:443-447.

327.   Millard JA, Hill BB, Cook PS, et al. Intermittent sequential pneumatic compression in prevention of venous stasis associated with pneumoperitoneum during laparoscopic cholecystectomy. *Arch Surg* 1993;128:914-918; discussion 918-919.

328.   Latimer FG, Eades SC, Pettifer G, et al. Cardiopulmonary, blood and peritoneal fluid alterations associated with abdominal insufflation of carbon dioxide in standing horses. *Equine Vet J* 2003;35:283-290.

329.   Donaldson LL, Trostle SS, White NA. Cardiopulmonary changes associated with abdominal insufflation of carbon dioxide in mechanically ventilated, dorsally recumbent, halothane anaesthetised horses. *Equine Vet J* 1998;30:144-151.

330.   Jones DR, Graeber GM, Tanguilig GG, et al. Effects of insufflation on hemodynamics during thoracoscopy. *Ann Thorac Surg* 1993;55:1379-1382.

331.   Ohtsuka T, Imanaka K, Endoh M, et al. Hemodynamic effects of carbon dioxide insufflation under single-lung ventilation during thoracoscopy. *Ann Thorac Surg* 1999;68:29-32; discussion 32-23.

332.   Polis I, Gasthuys F, Gielen I, et al. The effects of intrathoracic pressure during continuous two-lung ventilation for thoracoscopy on the cardiorespiratory parameters in sevoflurane anaesthetized dogs. *J Vet Med A Physiol Pathol Clin Med* 2002;49:113-120.

333.   Henneke DR, Potter GD, Kreider JL, et al. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* 1983;15:371-372.

334.   Bland JM, Altman DG. Agreement between methods of measurement with multiple observations per individual. *J Biopharm Stat* 2007;17:571-582.

335.   Gutt CN, Oniu T, Mehrabi A, et al. Circulatory and respiratory complications of carbon dioxide insufflation. *Dig Surg* 2004;21:95-105.

336.   Valenza F, Chevallard G, Porro GA, et al. Static and dynamic components of esophageal and central venous pressure during intra-abdominal hypertension. *Crit Care Med* 2007;35:1575-1581.

337.   Cicchetti DV. Multiple comparison methods: establishing guidelines for their valid application in neuropsychological research. *J Clin Exp Neuropsychol* 1994;16:155-161.

338.   Malhotra A, Cohen D, Syms C, et al. Blood pressure changes in the leg on standing. *J Clin Hypertens (Greenwich)* 2002;4:350-354.

339.   Raisis AL, Young LE, Meire HB, et al. Variability of Doppler ultrasound measurements of hindlimb blood flow in conscious horses. *Equine Vet J* 2000;32:125-132.

340.   Raisis AL, Young LE, Meire HB, et al. Measurements of hindlimb blood flow recorded using Doppler ultrasound during administration of vasoactive agents in halothane-anesthetized horses. *Vet Radiol Ultrasound* 2000;41:64-72.

341.   Raisis AL, Young LE, Blissitt KJ, et al. Effect of a 30-minute infusion of dobutamine hydrochloride on hind limb blood flow and hemodynamics in halothane-anesthetized horses. *Am J Vet Res* 2000;61:1282-1288.

342.   Schmitt H. The pharmacology of clonidine and related products. *Antihypertensive agents*: Springer, 1977;299-396.

343.   Segarra FM, A.S.; Hanson, R.R. Effects of detomidine hydrochloride and butorphanol tartrate on intra-abdominal pressure and abdominal perfusion pressure in normal horses. Phi Zeta Research Emphasis Day 2013;23.

344.	Yamashita K, Tsubakishita S, Futaok S, et al. Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *J Vet Med Sci* 2000;62:1025-1032.

345.	Wagner AE, Muir WW, 3rd, Hinchcliff KW. Cardiovascular effects of xylazine and detomidine in horses. *Am J Vet Res* 1991;52:651-657.

346.	Kerr DD, Jones EW, Holbert D, et al. Comparison of the effects of xylazine and acetylpromazine maleate in the horse. *Am J Vet Res* 1972;33:777-784.

347.	Garner HE, Amend JF, Rosborough JP. Effects on Bay Va 1470 on cardiovascular parameters in ponies. *Vet Med Small Anim Clin* 1971;66:1016-1017 passim.

348.	Muir WW, Skarda RT, Milne DW. Evaluation of xylazine and ketamine hydrochloride for anesthesia in horses. *Am J Vet Res* 1977;38:195-201.

349.	Angus DC, Barnato AE, Bell D, et al. A systematic review and meta-analysis of early goal-directed therapy for septic shock: the ARISE, ProCESS and ProMISe Investigators. *Intensive Care Med* 2015;41:1549-1560.

350.	Zhang L, Zhu G, Han L, et al. Early goal-directed therapy in the management of severe sepsis or septic shock in adults: a meta-analysis of randomized controlled trials. *BMC Med* 2015;13:71.

351.	Cheatham ML, Block EF, Nelson LD, et al. Superior predictor of the hemodynamic response to fluid challenge in critically ill patients. *Chest* 1998;114:1226-1227.

352.	Michard F, Alaya S, Zarka V, et al. Global end-diastolic volume as an indicator of cardiac preload in patients with septic shock. *Chest* 2003;124:1900-1908.

353.	de Laet IE, Malbrain M. Current insights in intra-abdominal hypertension and abdominal compartment syndrome. *Med Intensiva* 2007;31:88-99.

354.	Fielding CL, Stolba DN. Pulse pressure variation and systolic pressure variation in horses undergoing general anesthesia. *J Vet Emerg Crit Care (San Antonio)* 2012;22:372-375.

355.	Cheatham ML. Abdominal compartment syndrome: pathophysiology and definitions. *Scand J Trauma Resusc Emerg Med* 2009;17:10.

356.	Strimbu K, Tavel JA. What are biomarkers? *Curr Opin HIV AIDS* 2010;5:463-466.

357.   Biomarkers Definitions Working Group F-N. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics* 2001;69:89-95.

358.   Biomarker Working Group F-N. BEST (Biomarkers, EndpointS, and other Tools) Resource In: (US) FaDA, ed. Bethesda, MD: National Institutes of Health (US), 2016.

359.   Larsdotter S, Nostell K, von Euler H. Serum thymidine kinase activity in clinically healthy and diseased horses: a potential marker for lymphoma. *Vet J* 2015;205:313-316.

360.   Bullone M, de Lagarde M, Vargas A, et al. Serum Surfactant Protein D and Haptoglobin as Potential Biomarkers for Inflammatory Airway Disease in Horses. *J Vet Intern Med* 2015;29:1707-1711.

361.   Vincze B, Gaspardy A, Kulcsar M, et al. Equine alpha-fetoprotein levels in Lipizzaner mares with normal pregnancies and with pregnancy loss. *Theriogenology* 2015;84:1581-1586.

362.   Dymock DC, Brown MP, Merritt KA, et al. Concentrations of stromal cell-derived factor-1 in serum, plasma, and synovial fluid of horses with osteochondral injury. *Am J Vet Res* 2014;75:722-730.

363.   Gilliam LL, Holbrook TC, Ownby CL, et al. Cardiotoxicity, inflammation, and immune response after rattlesnake envenomation in the horse. *J Vet Intern Med* 2012;26:1457-1463.

364.   Nath LC, Anderson GA, Hinchcliff KW, et al. Serum cardiac troponin I concentrations in horses with cardiac disease. *Aust Vet J* 2012;90:351-357.

365.   Peters ST, Hopkins A, Stewart S, et al. Myocardial contusion and rib fracture repair in an adult horse. *J Vet Emerg Crit Care (San Antonio)* 2013;23:663-669.

366.   Nath LC, Anderson GA, Hinchcliff KW, et al. Clinicopathologic evidence of myocardial injury in horses with acute abdominal disease. *J Am Vet Med Assoc* 2012;241:1202-1208.

367.   Pihl TH, Andersen PH, Kjelgaard-Hansen M, et al. Serum amyloid A and haptoglobin concentrations in serum and peritoneal fluid of healthy horses and horses with acute abdominal pain. *Vet Clin Pathol* 2013;42:177-183.

368.   Westerman TL, Tornquist SJ, Foster CM, et al. Evaluation of serum amyloid A and haptoglobin concentrations as prognostic indicators for horses with inflammatory disease examined at a tertiary care hospital. *Am J Vet Res* 2015;76:882-888.

369.   Ludwig EK, Brandon Wiese R, Graham MR, et al. Serum and Synovial Fluid Serum Amyloid A Response in Equine Models of Synovitis and Septic Arthritis. *Vet Surg* 2016;45:859-867.

370.   Haglund U, Bulkley GB, Granger DN. On the pathophysiology of intestinal ischemic injury. Clinical review. *Acta Chir Scand* 1987;153:321-324.

371.   Patel A, Kaleya RN, Sammartano RJ. Pathophysiology of mesenteric ischemia. *Surg Clin North Am* 1992;72:31-41.

372.   Nielsen C, Kirkegard J, Erlandsen EJ, et al. D-lactate is a valid biomarker of intestinal ischemia induced by abdominal compartment syndrome. *J Surg Res* 2015;194:400-404.

373.   Duzgun AP, Gulgez B, Ozmutlu A, et al. The relationship between intestinal hypoperfusion and serum d-lactate levels during experimental intra-abdominal hypertension. *Dig Dis Sci* 2006;51:2400-2403.

374.   Yamout SZ, Nieto JE, Beldomenico PM, et al. Peritoneal and plasma D-lactate concentrations in horses with colic. *Vet Surg* 2011;40:817-824.

375.   Nappert G, Johnson PJ. Determination of the acid-base status in 50 horses admitted with colic between December 1998 and May 1999. *Can Vet J* 2001;42:703-707.

376.   Argyra E, Theodoraki K, Rellia P, et al. Atrial and brain natriuretic peptide changes in an experimental model of intra-abdominal hypertension. *J Surg Res* 2013;184:937-943.

377.   van der Vekens N, Decloedt A, de Clercq D, et al. Atrial natriuretic peptide vs. N-terminal-pro-atrial natriuretic peptide for the detection of left atrial dilatation in horses. *Equine Vet J* 2016;48:15-20.

378.   Bodnar Z, Keresztes T, Kovacs I, et al. Increased serum adenosine and interleukin 10 levels as new laboratory markers of increased intra-abdominal pressure. *Langenbecks Arch Surg* 2010;395:969-972.

379.   Brandon CI, Vandenplas M, Dookwah H, et al. Cloning and pharmacological characterization of the equine adenosine A2A receptor: a potential therapeutic target for the treatment of equine endotoxemia. *J Vet Pharmacol Ther* 2006;29:243-253.

380.   Brandon CI, Vandenplas M, Dookwah H, et al. Cloning and pharmacological characterization of the equine adenosine A3 receptor. *J Vet Pharmacol Ther* 2006;29:255-263.

381.	Fredholm BB, AP IJ, Jacobson KA, et al. International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001;53:527-552.

382.	Olah ME, Stiles GL. Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu Rev Pharmacol Toxicol* 1995;35:581-606.

383.	Sun WC, Berghaus LJ, Moore JN, et al. Lipopolysaccharide and TNF-alpha modify adenosine A(2A) receptor expression and function in equine monocytes. *Vet Immunol Immunopathol* 2010;135:289-295.

384.	Sun WC, Moore JN, Hurley DJ, et al. Adenosine A2A receptor agonists inhibit lipopolysaccharide-induced production of tumor necrosis factor-alpha by equine monocytes. *Vet Immunol Immunopathol* 2008;121:91-100.

385.	Bouma MG, Stad RK, van den Wildenberg FA, et al. Differential regulatory effects of adenosine on cytokine release by activated human monocytes. *J Immunol* 1994;153:4159-4168.

386.	Grisham MB, Hernandez LA, Granger DN. Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. *Am J Physiol* 1989;257:H1334-1339.

387.	Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* 2001;414:916-920.

388.	Lukashev D, Ohta A, Apasov S, et al. Cutting edge: Physiologic attenuation of proinflammatory transcription by the Gs protein-coupled A2A adenosine receptor in vivo. *J Immunol* 2004;173:21-24.

389.	Zarek PE, Huang CT, Lutz ER, et al. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. *Blood* 2008;111:251-259.

390.	Motew SJ, Mourelatos MG, Miller RN, et al. Evidence that adenosine contributes to maintenance of hepatosplanchnic blood flow during peritoneal sepsis in rats. *Shock* 1997;7:439-446.

391.	Bodnar Z, Szentkereszty Z, Hajdu Z, et al. Beneficial effects of theophylline infusions in surgical patients with intra-abdominal hypertension. *Langenbecks Arch Surg* 2011;396:793-800.

392.	Hansen PB, Hashimoto S, Oppermann M, et al. Vasoconstrictor and vasodilator effects of adenosine in the mouse kidney due to preferential activation of A1 or A2 adenosine receptors. *J Pharmacol Exp Ther* 2005;315:1150-1157.

393. De Waele JJ, De Laet I. Intra-abdominal hypertension and the effect on renal function. *Acta Clin Belg* 2007;62 Suppl 2:371-374.

394. Sui F, Zheng Y, Li WX, et al. Renal circulation and microcirculation during intra-abdominal hypertension in a porcine model. *Eur Rev Med Pharmacol Sci* 2016;20:452-461.

395. Wauters J, Claus P, Brosens N, et al. Pathophysiology of renal hemodynamics and renal cortical microcirculation in a porcine model of elevated intra-abdominal pressure. *J Trauma* 2009;66:713-719.

396. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy--review of a new approach. *Pharmacol Rev* 2003;55:241-269.

397. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008;180:5771-5777.

398. Moore KW, de Waal Malefyt R, Coffman RL, et al. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001;19:683-765.

399. Takanaski S, Nonaka R, Xing Z, et al. Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. *J Exp Med* 1994;180:711-715.

400. Arock M, Zuany-Amorim C, Singer M, et al. Interleukin-10 inhibits cytokine generation from mast cells. *Eur J Immunol* 1996;26:166-170.

401. de Waal Malefyt R, Abrams J, Bennett B, et al. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991;174:1209-1220.

402. Niiro H, Otsuka T, Izuhara K, et al. Regulation by interleukin-10 and interleukin-4 of cyclooxygenase-2 expression in human neutrophils. *Blood* 1997;89:1621-1628.

403. Monneret G, Finck ME, Venet F, et al. The anti-inflammatory response dominates after septic shock: association of low monocyte HLA-DR expression and high interleukin-10 concentration. *Immunol Lett* 2004;95:193-198.

404. Sherry RM, Cue JI, Goddard JK, et al. Interleukin-10 is associated with the development of sepsis in trauma patients. *J Trauma* 1996;40:613-616; discussion 616-617.

405. Giannoudis PV, Smith RM, Perry SL, et al. Immediate IL-10 expression following major orthopaedic trauma: relationship to anti-inflammatory response and subsequent development of sepsis. *Intensive Care Med* 2000;26:1076-1081.

406.   Ayala A, Lehman DL, Herdon CD, et al. Mechanism of enhanced susceptibility to sepsis following hemorrhage. Interleukin-10 suppression of T-cell response is mediated by eicosanoid-induced interleukin-4 release. *Arch Surg* 1994;129:1172-1178.

407.   Kobayashi H, Kobayashi M, Herndon DN, et al. Susceptibility of thermally injured mice to cytomegalovirus infection. *Burns* 2001;27:675-680.

408.   Kwon S, Moore JN, Robertson TP, et al. Disparate effects of LPS infusion and carbohydrate overload on inflammatory gene expression in equine laminae. *Vet Immunol Immunopathol* 2013;155:1-8.

409.   Lopes MA, Salter CE, Vandenplas ML, et al. Expression of genes associated with inflammation induced by ex vivo exposure to lipopolysaccharide in peripheral blood leukocytes from horses with gastrointestinal disease. *Am J Vet Res* 2010;71:1162-1169.

410.   Vinther AM, Skovgaard K, Heegaard PM, et al. Dynamic expression of leukocyte innate immune genes in whole blood from horses with lipopolysaccharide-induced acute systemic inflammation. *BMC Vet Res* 2015;11:134.

411.   Lopes MA, Salter CE, Vandenplas ML, et al. Expression of inflammation-associated genes in circulating leukocytes collected from horses with gastrointestinal tract disease. *Am J Vet Res* 2010;71:915-924.

412.   Hawkins DL, MacKay RJ, MacKay SL, et al. Human interleukin 10 suppresses production of inflammatory mediators by LPS-stimulated equine peritoneal macrophages. *Vet Immunol Immunopathol* 1998;66:1-10.

413.   Gerard C, Bruyns C, Marchant A, et al. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J Exp Med* 1993;177:547-550.

414.   Marchant A, Bruyns C, Vandenabeele P, et al. Interleukin-10 controls interferon-gamma and tumor necrosis factor production during experimental endotoxemia. *Eur J Immunol* 1994;24:1167-1171.

415.   Bonelli F, Meucci V, Divers TJ, et al. Kinetics of plasma procalcitonin, soluble CD14, CCL2 and IL-10 after a sublethal infusion of lipopolysaccharide in horses. *Vet Immunol Immunopathol* 2017;184:29-35.

416.   Burton AB, Wagner B, Erb HN, et al. Serum interleukin-6 (IL-6) and IL-10 concentrations in normal and septic neonatal foals. *Vet Immunol Immunopathol* 2009;132:122-128.

417.	Pusterla N, Magdesian KG, Mapes S, et al. Expression of molecular markers in blood of neonatal foals with sepsis. *Am J Vet Res* 2006;67:1045-1049.

418.	Sponseller BA, de Macedo MM, Clark SK, et al. Activation of peripheral blood monocytes results in more robust production of IL-10 in neonatal foals compared to adult horses. *Vet Immunol Immunopathol* 2009;127:167-173.

419.	Le Moine O, Stordeur P, Schandene L, et al. Adenosine enhances IL-10 secretion by human monocytes. *J Immunol* 1996;156:4408-4414.

420.	Nieto JE, Aldridge BM, Beldomenico PM, et al. Characterization of equine intestinal fatty acid binding protein and its use in managing horses with colic. *Am J Vet Res* 2005;66:223-232.

421.	Alpers DH, Bass NM, Engle MJ, et al. Intestinal fatty acid binding protein may favor differential apical fatty acid binding in the intestine. *Biochim Biophys Acta* 2000;1483:352-362.

422.	Lieberman JM, Sacchettini J, Marks C, et al. Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery* 1997;121:335-342.

423.	Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. *Clin Chim Acta* 2005;352:15-35.

424.	Matsumoto S, Sekine K, Funaoka H, et al. Diagnostic performance of plasma biomarkers in patients with acute intestinal ischaemia. *Br J Surg* 2014;101:232-238.

425.	Wiercinska-Drapalo A, Jaroszewicz J, Siwak E, et al. Intestinal fatty acid binding protein (I-FABP) as a possible biomarker of ileitis in patients with ulcerative colitis. *Regul Pept* 2008;147:25-28.

426.	Schurink M, Kooi EM, Hulzebos CV, et al. Intestinal fatty acid-binding protein as a diagnostic marker for complicated and uncomplicated necrotizing enterocolitis: a prospective cohort study. *PLoS One* 2015;10:e0121336.

427.	Kittaka H, Akimoto H, Takeshita H, et al. Usefulness of intestinal fatty acid-binding protein in predicting strangulated small bowel obstruction. *PLoS One* 2014;9:e99915.

428.	Kanda T, Tsukahara A, Ueki K, et al. Diagnosis of ischemic small bowel disease by measurement of serum intestinal fatty acid-binding protein in patients with acute abdomen: a multicenter, observer-blinded validation study. *J Gastroenterol* 2011;46:492-500.

429.   Shi H, Wu B, Wan J, et al. The role of serum intestinal fatty acid binding protein levels and D-lactate levels in the diagnosis of acute intestinal ischemia. *Clin Res Hepatol Gastroenterol* 2015;39:373-378.

430.   Cronk DR, Houseworth TP, Cuadrado DG, et al. Intestinal fatty acid binding protein (I-FABP) for the detection of strangulated mechanical small bowel obstruction. *Curr Surg* 2006;63:322-325.

431.   Timmermans K, Sir O, Kox M, et al. Circulating iFABP Levels as a marker of intestinal damage in trauma patients. *Shock* 2015;43:117-120.

432.   Bingold TM, Franck K, Holzer K, et al. Intestinal Fatty Acid Binding Protein: A Sensitive Marker in Abdominal Surgery and Abdominal Infection. *Surg Infect (Larchmt)* 2015;16:247-253.

433.   Piton G, Belon F, Cypriani B, et al. Enterocyte damage in critically ill patients is associated with shock condition and 28-day mortality. *Crit Care Med* 2013;41:2169-2176.

434.   Caspeta L, Nieto I, Zamilpa A, et al. Solanum chrysotrichum hairy root cultures: characterization, scale-up and production of five antifungal saponins for human use. *Planta Med* 2005;71:1084-1087.

435.   Strang SG, Van Waes OJ, Van der Hoven B, et al. Intestinal fatty acid binding protein as a marker for intra-abdominal pressure-related complications in patients admitted to the intensive care unit; study protocol for a prospective cohort study (I-Fabulous study). *Scand J Trauma Resusc Emerg Med* 2015;23:6.

436.   Meisner M. Update on procalcitonin measurements. *Ann Lab Med* 2014;34:263-273.

437.   Toribio RE, Kohn CW, Leone GW, et al. Molecular cloning and expression of equine calcitonin, calcitonin gene-related peptide-I, and calcitonin gene-related peptide-II. *Mol Cell Endocrinol* 2003;199:119-128.

438.   Muller B, Becker KL. Procalcitonin: how a hormone became a marker and mediator of sepsis. *Swiss Med Wkly* 2001;131:595-602.

439.   Hoffmann G, Totzke G, Seibel M, et al. In vitro modulation of inducible nitric oxide synthase gene expression and nitric oxide synthesis by procalcitonin. *Crit Care Med* 2001;29:112-116.

440.   Hoffmann G, Czechowski M, Schloesser M, et al. Procalcitonin amplifies inducible nitric oxide synthase gene expression and nitric oxide production in vascular smooth muscle cells. *Crit Care Med* 2002;30:2091-2095.

441.   Wrenger S, Kahne T, Bohuon C, et al. Amino-terminal truncation of procalcitonin, a marker for systemic bacterial infections, by dipeptidyl peptidase IV (DP IV). *FEBS Lett* 2000;466:155-159.

442.   Monneret G, Pachot A, Laroche B, et al. Procalcitonin and calcitonin gene-related peptide decrease LPS-induced tnf production by human circulating blood cells. *Cytokine* 2000;12:762-764.

443.   Wiedermann FJ, Kaneider N, Egger P, et al. Migration of human monocytes in response to procalcitonin. *Crit Care Med* 2002;30:1112-1117.

444.   Meisner M, Tschaikowsky K, Hutzler A, et al. Postoperative plasma concentrations of procalcitonin after different types of surgery. *Intensive Care Med* 1998;24:680-684.

445.   Meisner M, Schmidt J, Huttner H, et al. The natural elimination rate of procalcitonin in patients with normal and impaired renal function. *Intensive Care Med* 2000;26 Suppl 2:S212-216.

446.   Copp DH, Cheney B. Calcitonin-a hormone from the parathyroid which lowers the calcium-level of the blood. *Nature* 1962;193:381-382.

447.   Baruti Gafurri Z, Pacarizi H, Zhubi B, et al. The importance of determining procalcitonin and C reactive protein in different stages of sepsis. *Bosn J Basic Med Sci* 2010;10:60-64.

448.   Becker KL, Nylen ES, White JC, et al. Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. *J Clin Endocrinol Metab* 2004;89:1512-1525.

449.   Muller B, Christ-Crain M, Nylen ES, et al. Limits to the use of the procalcitonin level as a diagnostic marker. *Clin Infect Dis* 2004;39:1867-1868.

450.   Carboni GL, Fahrner R, Gazdhar A, et al. Comparison of procalcitonin and CrP in the postoperative course after lung decortication. *Eur J Cardiothorac Surg* 2008;33:777-780.

451.   Cosse C, Sabbagh C, Kamel S, et al. Procalcitonin and intestinal ischemia: a review of the literature. *World J Gastroenterol* 2014;20:17773-17778.

452.   Meisner M. Pathobiochemistry and clinical use of procalcitonin. *Clin Chim Acta* 2002;323:17-29.

453.   Kiriyama Y, Nomura Y, Tokumitsu Y. Calcitonin gene expression induced by lipopolysaccharide in the rat pituitary. *Am J Physiol Endocrinol Metab* 2002;282:E1380-1384.

454.   Muller B, Becker KL, Kranzlin M, et al. Disordered calcium homeostasis of sepsis: association with calcitonin precursors. *Eur J Clin Invest* 2000;30:823-831.

455.   Oberhoffer M, Stonans I, Russwurm S, et al. Procalcitonin expression in human peripheral blood mononuclear cells and its modulation by lipopolysaccharides and sepsis-related cytokines in vitro. *J Lab Clin Med* 1999;134:49-55.

456.   Assicot M, Gendrel D, Carsin H, et al. High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet* 1993;341:515-518.

457.   Cosse C, Sabbagh C, Browet F, et al. Serum value of procalcitonin as a marker of intestinal damages: type, extension, and prognosis. *Surg Endosc* 2015;29:3132-3139.

458.   Stolberg L, Rolfe R, Gitlin N, et al. d-Lactic acidosis due to abnormal gut flora: diagnosis and treatment of two cases. *N Engl J Med* 1982;306:1344-1348.

459.   Lavrentieva A, Kontakiotis T, Lazaridis L, et al. Inflammatory markers in patients with severe burn injury. What is the best indicator of sepsis? *Burns* 2007;33:189-194.

460.   Nijsten MW, Olinga P, The TH, et al. Procalcitonin behaves as a fast responding acute phase protein in vivo and in vitro. *Crit Care Med* 2000;28:458-461.

461.   Bracq S, Machairas M, Clement B, et al. Calcitonin gene expression in normal human liver. *FEBS Lett* 1993;331:15-18.

462.   Redl H, Schlag G, Togel E, et al. Procalcitonin release patterns in a baboon model of trauma and sepsis: relationship to cytokines and neopterin. *Crit Care Med* 2000;28:3659-3663.

463.   Meisner M, Muller V, Khakpour Z, et al. Induction of procalcitonin and proinflammatory cytokines in an anhepatic baboon endotoxin shock model. *Shock* 2003;19:187-190.

464.   Nylen ES, Whang KT, Snider RH, Jr., et al. Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. *Crit Care Med* 1998;26:1001-1006.

465.   Martinez JM, Wagner KE, Snider RH, et al. Late immunoneutralization of procalcitonin arrests the progression of lethal porcine sepsis. *Surg Infect (Larchmt)* 2001;2:193-202; discussion 202-193.

466.   Nylen ES, Snider RH, Jr., Thompson KA, et al. Pneumonitis-associated hyperprocalcitoninemia. *Am J Med Sci* 1996;312:12-18.

467.   Davis TM, Li GQ, Guo XB, et al. Serum ionized calcium, serum and intracellular phosphate, and serum parathormone concentrations in acute malaria. *Trans R Soc Trop Med Hyg* 1993;87:49-53.

468.   Dandona P, Nix D, Wilson MF, et al. Procalcitonin increase after endotoxin injection in normal subjects. *J Clin Endocrinol Metab* 1994;79:1605-1608.

469.   Adamik B, Kubler-Kielb J, Golebiowska B, et al. Effect of sepsis and cardiac surgery with cardiopulmonary bypass on plasma level of nitric oxide metabolites, neopterin, and procalcitonin: correlation with mortality and postoperative complications. *Intensive Care Med* 2000;26:1259-1267.

470.   Eberhard OK, Langefeld I, Kuse ER, et al. Procalcitonin in the early phase after renal transplantation--will it add to diagnostic accuracy? *Clin Transplant* 1998;12:206-211.

471.   Costa MC, Silva G, Ramos RV, et al. Characterization and comparison of the bacterial microbiota in different gastrointestinal tract compartments in horses. *Vet J* 2015;205:74-80.

472.   Wacker C, Prkno A, Brunkhorst FM, et al. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *Lancet Infect Dis* 2013;13:426-435.

473.   Moore JN, Vandenplas ML. Is it the systemic inflammatory response syndrome or endotoxemia in horses with colic? *Vet Clin North Am Equine Pract* 2014;30:337-351, vii-viii.

474.   Bonelli F, Meucci V, Divers TJ, et al. Plasma Procalcitonin Concentration in Healthy Horses and Horses Affected by Systemic Inflammatory Response Syndrome. *J Vet Intern Med* 2015;29:1689-1691.

475.   Corley KT, Donaldson LL, Furr MO. Arterial lactate concentration, hospital survival, sepsis and SIRS in critically ill neonatal foals. *Equine Vet J* 2005;37:53-59.

476.   Bonelli F, Meucci V, Divers T, et al. Evaluation of Plasma Procalcitonin Concentrations in Healthy Foals and Foals Affected by Septic Systemic Inflammatory Response Syndrome. *Journal of Equine Veterinary Science* 2015;35:645-649.

477.   Altunhan H, Annagur A, Ors R, et al. Procalcitonin measurement at 24 hours of age may be helpful in the prompt diagnosis of early-onset neonatal sepsis. *Int J Infect Dis* 2011;15:e854-858.

478.   Lacour AG, Gervaix A, Zamora SA, et al. Procalcitonin, IL-6, IL-8, IL-1 receptor antagonist and C-reactive protein as identificators of serious bacterial infections in children with fever without localising signs. *Eur J Pediatr* 2001;160:95-100.

479.   Yilmaz Z, Ilcol YO, Ulus IH. Endotoxin increases plasma leptin and ghrelin levels in dogs. *Crit Care Med* 2008;36:828-833.

480.   Cosse C, Regimbeau JM, Fuks D, et al. Serum procalcitonin for predicting the failure of conservative management and the need for bowel resection in patients with small bowel obstruction. *J Am Coll Surg* 2013;216:997-1004.

481.   Vidal MG, Ruiz Weisser J, Gonzalez F, et al. Incidence and clinical effects of intra-abdominal hypertension in critically ill patients. *Crit Care Med* 2008;36:1823-1831.

482.   Diebel L, Saxe J, Dulchavsky S. Effect of intra-abdominal pressure on abdominal wall blood flow. *Am Surg* 1992;58:573-575; discussion 575-576.

483.   Frellstedt L, McKenzie HC, Barrett JG, et al. Induction and characterization of endotoxin tolerance in equine peripheral blood mononuclear cells in vitro. *Vet Immunol Immunopathol* 2012;149:97-102.

484.   Kox M, de Kleijn S, Pompe JC, et al. Differential ex vivo and in vivo endotoxin tolerance kinetics following human endotoxemia. *Crit Care Med* 2011;39:1866-1870.

485.   Kaikaus RM, Bass NM, Ockner RK. Functions of fatty acid binding proteins. *Experientia* 1990;46:617-630.

486.   Rieger M, Kochleus C, Teschner D, et al. A new ELISA for the quantification of equine procalcitonin in plasma as potential inflammation biomarker in horses. *Anal Bioanal Chem* 2014;406:5507-5512.

487.   Twohy CW, Duran AP, Munson TE. Endotoxin contamination of parenteral drugs and radiopharmaceuticals as determined by the limulus amebocyte lysate method. *J Parenter Sci Technol* 1984;38:190-201.

488.   Nylen ES, Jeng J, Jordan MH, et al. Late pulmonary sequela following burns: persistence of hyperprocalcitonemia using a 1-57 amino acid N-terminal flanking peptide assay. *Respir Med* 1995;89:41-46.

489.   Campos-Munoz MA, Villarreal-Rios E, Chimal-Torres M, et al. [Intra-abdominal pressure as a surgery predictor in patients with acute abdominal pain]. *Rev Med Inst Mex Seguro Soc* 2016;54:280-285.

490.   Guerrero-Romero F, Rodriguez-Moran M. Abdominal volume index. An anthropometry-based index for estimation of obesity is strongly related to impaired glucose tolerance and type 2 diabetes mellitus. *Arch Med Res* 2003;34:428-432.

491.   Abu-Rafea B, Vilos GA, Vilos AG, et al. Effect of body habitus and parity on insufflated CO_2 volume at various intraabdominal pressures during laparoscopic access in women. *J Minim Invasive Gynecol* 2006;13:205-210.

492.   McDougall EM, Figenshau RS, Clayman RV, et al. Laparoscopic pneumoperitoneum: impact of body habitus. *J Laparoendosc Surg* 1994;4:385-391.

493.   Cordemans C, De Laet I, Van Regenmortel N, et al. Fluid management in critically ill patients: the role of extravascular lung water, abdominal hypertension, capillary leak, and fluid balance. *Ann Intensive Care* 2012;2:S1.

494.   De Waele JJ, Hoste EA, Malbrain ML. Decompressive laparotomy for abdominal compartment syndrome--a critical analysis. *Crit Care* 2006;10:R51.

495.   Smith LJ, Mellor DJ, Marr CM, et al. Incisional complications following exploratory celiotomy: does an abdominal bandage reduce the risk? *Equine Vet J* 2007;39:277-283.

496.   Barrett EJ, Munsterman AS, Hanson RR. Effects of compressive abdominal bandaging and transrectal palpation on intra-abdominal pressures in horses. *J Vet Emerg Crit Care (San Antonio)* 2013;23:41-46.

497.   Rabe KF, Magnussen H, Dent G. Theophylline and selective PDE inhibitors as bronchodilators and smooth muscle relaxants. *Eur Respir J* 1995;8:637-642.

498.   Rickards KJ, Page CP, Lees P, et al. Differential inhibition of equine neutrophil function by phosphodiesterase inhibitors. *J Vet Pharmacol Ther* 2001;24:275-281.

499.   Culpitt SV, de Matos C, Russell RE, et al. Effect of theophylline on induced sputum inflammatory indices and neutrophil chemotaxis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;165:1371-1376.

500.   Tomita K, Chikumi H, Tokuyasu H, et al. Functional assay of NF-kappaB translocation into nuclei by laser scanning cytometry: inhibitory effect by dexamethasone or theophylline. *Naunyn Schmiedebergs Arch Pharmacol* 1999;359:249-255.

501.   Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1beta-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 2000;20:6891-6903.

502.   Pauwels RA, Joos GF. Characterization of the adenosine receptors in the airways. *Arch Int Pharmacodyn Ther* 1995;329:151-160.

503.   Osswald H, Schnermann J. Methylxanthines and the kidney. *Handb Exp Pharmacol* 2011:391-412.

504.   Benoehr P, Krueth P, Bokemeyer C, et al. Nephroprotection by theophylline in patients with cisplatin chemotherapy: a randomized, single-blinded, placebo-controlled trial. *J Am Soc Nephrol* 2005;16:452-458.

505.   Huber W, Eckel F, Hennig M, et al. Prophylaxis of contrast material-induced nephropathy in patients in intensive care: acetylcysteine, theophylline, or both? A randomized study. *Radiology* 2006;239:793-804.

506.   Kowalczyk DF, Beech J, Littlejohn D. Pharmacokinetic disposition of theophylline in horses after intravenous administration. *Am J Vet Res* 1984;45:2272-2275.

507.   Ingvast-Larsson C. Relaxant effects of theophylline and clenbuterol on tracheal smooth muscle from horse and rat in vitro. *J Vet Pharmacol Ther* 1991;14:310-316.

508.   Barnes PJ. Theophylline: new perspectives for an old drug. *Am J Respir Crit Care Med* 2003;167:813-818.