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Abstract approved:

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Abstract:

Lactate is an end-product of both aerobic and anaerobic glycolysis and it can be used as an energy source for tissues. It is produced by all cells in the mammalian body that can metabolize glucose. Increased production of lactate can be seen with there is an increase in cellular metabolism or a decrease in available oxygen to tissues. Therefore, it is a valuable marker in both human and veterinary medicine for many patients with critical illness and/or sepsis for monitoring response to treatment, severity of disease, and likelihood of survival or development of complications. In equine medicine, lactate production has been shown to vary between different regions of the body, however there is little information regarding production in the limbs of the horse and in its development secondary to tissue perfusion altering medications.

In the first study detailed here, we evaluated the differences between jugular and cephalic vein venous blood gas variables in both healthy and clinically ill horses, with a focus on lactate. We took simultaneous samples from both the jugular and cephalic veins in 10 healthy horses and compared these two sites to each other. We found that the cephalic vein lactate was significantly higher than the jugular vein lactate in these horses ($p<0.05$). Additionally, we took simultaneous jugular and cephalic vein samples from horses that presented to the Oregon State University, College of

Veterinary Medicine for potential colitis. With these horses, additional samples were obtained during the first 24-hours of hospitalization to monitor how these variables change with treatment. For all sick horses, the cephalic vein lactate was significantly higher than the jugular vein lactate at presentation and during all time points sampled after ($p<0.05$). When the sick horses were investigated for specific outcomes (survivors vs. non-survivors and laminitis vs. non-laminitis) the cephalic vein was significantly higher in survivors and horses that did not develop laminitis ($p<0.05$) however no significant difference was found in non-survivors or horses that developed laminitis. Additionally, no significant difference was seen in the rate of change in lactate over time when survivors were compared to non-survivors and when horses who developed laminitis were compared to those that did not.

In the second study, the main objective was to establish how perfusion parameters change in horses that undergo prolonged sedation with the α-2 agonist, detomidine. The primary variable examined was lactate, however attention was also given to venous oxygen and carbon dioxide content as well as to blood glucose. Statistically significant differences ($p<0.05$) were seen in all variables examined, however the only variable that changed in a manner that would be considered clinically significant was glucose, with a mean increase in circulating blood glucose of 156 mg/dL. This represents an almost 3-fold increase in circulating blood glucose measurements, which remained elevated throughout the course of the four-hour infusion. While lactate did not elevate above the normal threshold value (>2.0 mmol/L), these horses also did not achieve a clinically relevant plane of sedation for standing surgical procedures. Therefore, further study is warranted to assess how lactate may change in a more clinically relevant setting.

Clinical Applications of Lactate Measurements in Equine Veterinary Medicine.

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Rose E. Baker, Author

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1.1.1 Normal Cellular Metabolism

The primary products of carbohydrate digestion in horses and ruminants are glucose, galactose and fructose with glucose comprising approximately 80% of the total breakdown product [1]. Glucose is utilized intracellularly to generate adenosine triphosphate (ATP). If it is not utilized immediately upon entering the cell, it can be stored in the form of glycogen, a large glucose polymer [1]. All cells are capable of glycogen storage, but the primary cells responsible for this storage are found in skeletal muscle and the liver [1].

Glycogen is formed through the process of glycogenesis, starting when glucose in the blood stream enters the cell and is rapidly converted to glucose-6-phosphate. From there it undergoes a series of enzyme mediated reactions within the cell to form glycogen. Additionally, glycogen can be formed from other molecules including lactic acid, glycerol, pyruvic acid and deaminated amino acids via conversion into glucose or related compounds [1]. These pathways become more important during periods of excess or hypoglycemia [1].

Glycogen is broken down to form intracellular glucose by the process of glycogenolysis. This process involves phosphorylation of each individual glucose molecule on the glycogen polymer. Phosphorylase, the enzyme responsible for this

process, is activated by epinephrine or glucagon [1]. Epinephrine is released from the adrenal medulla in response to stimulation of the sympathetic nervous system and glucagon is released by alpha cells of the pancreas in response to hypoglycemia [1]. Both hormones result in formation of cyclic AMP, which activates the phosphorylase enzyme and initiates release of glucose from the polymer [1].

The freed glucose molecules then undergo glycolysis in the cytosol, the process by which a molecule of glucose is broken down into two molecules of pyruvic acid. Glycolysis results in the production of four moles of ATP for every one mole of glucose utilized. However, there is a net gain of only two moles of ATP due to utilization of ATP in the pathway [1]. The two molecules of pyruvic acid may be reversibly converted to lactate or migrate into the mitochondrion, where they combine with two molecules of coenzyme-A, forming acetyl coenzyme A (acetyl-CoA), two molecules of carbon dioxide and four molecules of hydrogen [1]. These four hydrogen molecules become important for generating ATP.

Acetyl-CoA then enters the Krebs cycle (citric acid cycle) in the mitochondrial matrix [1]. In this cycle, the acetyl portion of acetyl-CoA is broken down into carbon dioxide and hydrogen atoms. The hydrogen atoms are important in downstream reactions for the generation of ATP. The Krebs cycle is responsible for a net gain of two moles of ATP [1].

The main generation of ATP comes from oxidative phosphorylation. This is the pathway where previously liberated hydrogen atoms play a vital role in the production of almost 90% of the ATP generated through glucose metabolism [1]. In this process, pairs of hydrogen atoms previously released become ionized through binding

of one atom with NAD^+, forming NADH and ionic hydrogen [1]. The free hydrogen and the hydrogen ion in the NADH each release an electron. The two electrons then enter the electron transport chain along the inner membrane of the mitochondria [1]. The electrons are shuttled downstream until they reach cytochrome A3 (cytochrome oxidase), which releases two electrons in order to reduce elemental oxygen, followed by combination with the previously formed hydrogen ions to form water [1]. The transport of the electrons along the electron transport chain results in a large release of energy at each step that is used to move hydrogen ions from the inner matrix of the mitochondria into the outer chamber (between the inner and outer mitochondrial membranes) [1]. This establishes a strong electrical gradient between these two potential spaces. The hydrogen ions then flow down their electrical gradient through a large transmembrane protein molecule, ATP synthetase (ATPase), and then back into the inner matrix [1]. The energy derived from the flow of these ions through ATPase is used to form ATP from ADP and free ionic phosphate radical (Pi) [1]. The combined effort of all of these steps results in a net formation of 36 moles of ATP for every mole of glucose degraded [1].

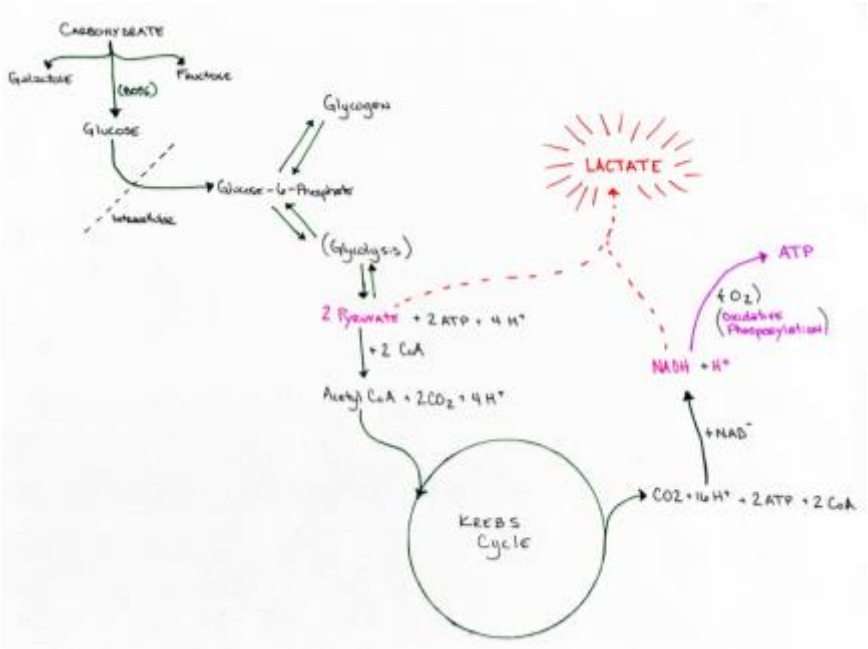
1.1.2 Lactic Acid Production in Cellular Metabolism

The previously described oxidative phosphorylation pathway, where much of the cells ATP is produced, is reliant on oxygen as the final electron accepter. When there is insufficient oxygen for this pathway, ATP generation relies solely on the glycolytic pathways without the addition of oxidative phosphorylation [1]. This is an

inefficient process, however, in that only 3% of the total potential energy from a glucose molecule is used to generate ATP [1]. As discussed earlier, the end products of glycolysis are two molecules of pyruvic acid and two hydrogen atoms, with the hydrogen atoms combining with NAD^+ to form NADH and H^+ [1]. If oxidative phosphorylation is impaired, excessive amounts of these glycolytic end products accumulate. In order to prevent slowing of glycolysis due to an accumulation of substrate pyruvate will combine with NADH and H^+, in the presence of lactate dehydrogenase to form lactic acid [1]. Therefore, lactic acid is the major end product of anaerobic glycolysis and it is freely diffusible from the cells of production into the extracellular fluid and other cells [1]. This prevents the accumulation of glycolysis substrates, allowing the reactions to continue. It is important to note that lactic acid is also an intermediate product of both aerobic glycolysis, and that some cells (mainly erythrocytes) are incapable of oxidative phosphorylation, so even in normal, healthy mammals a small amount of lactate is consistently produced [2,3]. The normal rate of lactic acid production in humans is estimated at 0.8-1 mmol/kg/hr [2,4]. Under conditions of anaerobic glycolysis, lactic acid production is increased. This product readily dissociates into lactate and hydrogen ions, with the hydrogen ions ultimately entering the oxidative phosphorylation pathway [3]. The concentration of lactate in the cytosol of a cell at any given time is controlled primarily by the NADH:NAD^+ ratio, the concentration of pyruvic acid and the concentration of hydrogen ions [3]. Increases in NADH or pyruvic acid and decreases in hydrogen ions will generate an increase in production of lactic acid [3]. The primary sites of lactic acid production under normal conditions in humans include the skeletal muscle, brain, and erythrocyte mass [2].

Other, sites of production include leukocytes, platelets, and the renal medulla [2]. Figure 1 summarizes the flow of carbohydrate metabolism.

Figure 1: Carbohydrate Metabolism & Lactate Production



1.1.3 In Vivo Lactate Utilization and Metabolism

From an in vivo perspective, the terms lactic acid and pyruvic acid are inappropriate. This is because, under normal physiologic conditions, the pK of both compounds is significantly lower than the body's pH, so these two compounds will readily dissociate into a hydrogen ion and lactate and pyruvate, respectively [2,3]. More than 99% of the lactic acid produced in the body dissociates, roughly to a ratio of about 3,000:1 [2,3,5]. Therefore, the correct terminology for each molecule under physiologic conditions will be utilized for the remainder of the discussion.

Following a period of anaerobic glycolysis when oxygen again becomes available, the previously formed lactate is re-converted to pyruvate, NADH and H^+. The NADH and H^+ is then cycled into the oxidative phosphorylation pathway and the remaining excess pyruvate is converted to glucose through the Cori cycle. Therefore, most of the lactate formed in the body is not lost. The primary site for this reconversion is the liver, with approximately 50% of lactate utilization occurring here [3]. Other tissues, primarily the kidney (30%) and skeletal muscle, also have the capability for lactate utilization through the Cori cycle [3,6]. The kidney also has the ability to excrete a very small amount of lactate [7]. In humans, the renal threshold for lactate excretion is 6-10 mM and under normal conditions <2% is excreted in the urine [7,8]. Additionally, there is evidence lactate is shuttled from one tissue to another for utilization [5,9]. For example, during moderate to high intensity exercise cardiac muscle is especially adept at converting lactate produced specifically from skeletal muscle to pyruvate and then utilizing it for energy stores [5,9]. Despite the liver being responsible for a large portion of lactate metabolism, liver dysfunction alone will not result in significant hyperlactemia due to recruitment of other tissues, and elevations in systemic lactate will not be seen until at least 70% of hepatic blood flow is lost [3,10].

The full intracellular role of lactate production and utilization is still unclear. Traditionally, with intracellular production, the belief was that lactate was only utilized to replenish NAD^+ and any remainder was then excreted for use in other cells [11]. However, more recent research has demonstrated that lactate enters into the mitochondria of the cell where it was produced to be used as an energy source for that cell [11]. This has especially important implications in rapidly growing and dividing

cells, such a neoplastic cells because lactate could be an important source of energy for cellular growth and serve as a potential target of anti-neoplastic therapy [11].

When illustrating the pathway of anaerobic glycolysis, the classic model utilized is hypoxia. In situations of hypoxia, when there is limited oxygen available for oxidative phosphorylation, the anaerobic glycolytic pathway and the reduction of pyruvate to lactate is essential for cell survival [1]. With severe and prolonged hypoxia or dysoxia, significant production of lactic acid can lead to metabolic acidosis due to the excessive production of hydrogen ions and decreased ATP production [2].

Lactate production increases under several physiologic conditions in healthy animals. The most common model of normal increases in lactate production is high intensity exercise. The mechanisms by which lactate production is increased during exercise are based on oxygen-dependent metabolism, accelerated glycolysis, decreased lactate removal and recruitment of lactate producing cells [5]. During aerobic metabolism, as exercise intensity increases, the partial pressure of oxygen in exercising muscle decreases, and more ATP is cleaved to provide energy to the cell. To regenerate ATP, greater amounts of ADP stimulate more oxidative phosphorylation [5]. Glycolysis also accelerates, and as oxygen demand outstrips supply, this results in increased lactate production [5]. The rate of glycolysis also becomes accelerated during this process by the activation of Na-K-ATPase from circulating catecholamines, especially epinephrine [5]. The increased activity of the Na-K-ATPase leads to increased lactate production due to the association of the enzyme with other compartmentalized glycolytic enzymes [12]. Additionally, lactate

producing cells, such as fast-twitch muscle fibers can produce lactate during exercise at a rate that exceeds metabolism, resulting in a net gain of lactate [5].

Lactate production in these tissues with a higher rate of production can serve as a source of lactate for their own metabolic purposes via the intracellular lactate shuttle or for other tissues in the body by the cell-to-cell lactate shuttle [5,11]. With the intracellular lactate shuttle, lactate produced within the cell is able to be utilized within the same cell for its own metabolic needs [5]. A recent study has shown that lactate dehydrogenase associated with mitochondria is capable of oxidizing lactate produced within the cell to pyruvate within the organelle [11]. This mitochondrial lactate dehydrogenase allows the cell to make use of its own lactate as an energy source by shuttling lactate from the cytosol into the mitochondria for oxidation [11]. Another significant theory on the utilization of lactate within the body is the cell-to-cell lactate shuttle. During periods of increase lactate production, especially in tissues that can significant increase their production such as skeletal muscle, lactate production can exceed clearance or utilization [13,14]. This excess lactate diffuses into the neighboring oxidative muscle fibers or into the systemic circulation for use in other tissues, which can oxidize the lactate [5,13]. Some lactate generated in one tissue is utilized specifically by another tissues [5]. For example, lactate generated from skeletal muscle has been shown to be taken up by other highly oxidative tissues, such as the brain and heart [5,13]. This data demonstrates that lactate is an easily transferrable substrate of glucose metabolism that is readily transported within cells as well as to nearby and distant tissues.

1.1.4 Effect of Critical Illness and Sepsis on Lactate

In critical illness, regular homeostatic mechanisms are frequently disrupted as part of the disease process, and this disruption plays a significant role in the development of a variety of measurable physiological disorders. Regarding lactate kinetics, many abnormalities arise because of aberrations in the circulatory system, tissue hypoxia, hypermetabolic states, endogenous hormone or exogenous medication administration and organ dysfunction. These aberrations result in the body either overproducing lactate under favorable conditions or being unable to successfully metabolize the lactate produced. If either one of these conditions is encountered then hyperlactemia results and commonly from both overproduction and lack of metabolism, which are present in varying degrees of severity. Hyperlactemia differs from lactic acidosis. Hyperlactemia is commonly seen when there are mild to moderate increases in lactate, generally <4 mmol/L, without any associated changes to pH [15,16]. Lactic acidosis, on the other hand, is defined as a moderate to severe elevation in serum lactate, generally >5 mmol/L, in conjunction with a decreased pH (<7.35) [16]. Due to compensatory mechanisms of the body for mild to moderate elevations in lactate, hyperlactemia is much more commonly seen under clinical conditions with lactic acidosis primarily occurring in severe illness and sepsis [6,16].

When tissue hypoxia occurs in the shock state, hyperlactemia develops. Under shock conditions, tissue hypoxia usually is a result of tissue hypoperfusion [17]. Both cardiogenic and hypovolemic shock are the most straight forward regarding their lactate formation under hypoxic conditions. Both types of shock are manifested by

decreased cardiac output, causing decreased perfusion and oxygen delivery to tissues. Tissue hypoxia leads to anaerobic glycolysis and the generation of lactate [2,3,17]. However, tissue hypoxia is only a small contributor to the development of systemic hyperlactemia and lactic acidosis in most cases of clinical illness [6,12,16]. So, while hypoperfusion plays a role in the generation of lactate in critically ill patient, it is not the sole source and this should be considered.

One of the early indicators that lactate was not strictly a measure of tissue hypoxia was the appreciation that in many critical illnesses, especially sepsis, the severity of hyperlactemia is generally incongruent with the degree of tissue hypoxia but congruent with the severity of disease [18]. Research at the time demonstrated, through the administration of dichloroacetate (DCA), that decreased oxygen delivery is not the major source of lactate production in these patients [18]. Dichloroacetate is a compound that stimulates pyruvate dehydrogenase to increase pyruvate oxidation [19]. Therefore, if oxygen deficiency during sepsis is the source of increased production, then administering DCA would not have any effect on the rate of pyruvate oxidation [18]. However, administration of DCA to septic human patients in this study significantly increased pyruvate oxidation, resulting in reduced glucose and lactate production [18]. This suggested that oxygen deficiency was not the cause of the hyperlactemia and led to the investigation of other mechanisms for lactate production [18].

One of the first hypotheses investigated was that increased lactate production was a result of decreased rate of pyruvate reduction. These early studies also found that sepsis, but not sterile inflammation, altered the rate of pyruvate dehydrogenase

activity [20]. This increased rate resulted from a three-fold decrease in the pyruvate dehydrogenase complex, resulting in an increased amount of pyruvate that was converted to lactate [20]. Additionally, it was also noted that the rate of glycolysis was increased in these subjects as well [18,20]. Specifically, septic patients had a rate of pyruvate production and oxidation that was 450% higher than controls [18]. Therefore, it appears, at least in part, that lactate elevations appreciated in septic patients are related to elevated pyruvate production potentially from impaired utilization. The authors postulated this elevated lactate would be a benefit to these patients because the more acidic environment generated by lactic acid production and its subsequent dissociation into lactate and hydrogen ions would encourage more rapid dissociation of oxygen from hemoglobin and therefore improve tissue oxygen delivery overall [18].

The lungs can act as another source of lactate under septic conditions and is most commonly seen in acute lung injury (ALI) or acute respiratory distress syndrome (ARDS). In the normal lung, no net release of lactate is appreciated because the rate of production is equal to the rate of utilization in the tissues [21,22]. However, in patients with ALI/ARDS lactate production exceeds utilization [23]. Specifically, in septic ALI/ARDS, even when there is not direct lung injury and the oxygen delivery to the lung is normal, they can still be a significant source of lactate production [22]. However, this elevation in lactate is not seen in other types of respiratory disease [23]. Therefore, it appears that lung tissue may therefore upregulate production of lactate in response to systemic mediators such as cytokines, endotoxin and infiltrating inflammatory cells, even when there is not direct injury to the lung tissue itself [22].

Lactate production is not restricted to periods of hypoxia. It can occur in patients with sepsis and critical illness, such as burns and trauma, under aerobic conditions. Sepsis and endotoxemia can stimulate upregulation of the GLUT-1 glucose transporter in tissues, increasing the influx of glucose and therefore increased pyruvate production and therefore lactate production as well [24]. Additionally, evidence exists that supports the theory that circulating catecholamines, specifically epinephrine are released during sepsis, head trauma and hemorrhagic shock, stimulating Na-K-ATPase-coupled lactate production in skeletal muscle [12,25,26]. This results in increased pyruvate production and oxidation [25]. Lactate concentrations are highest in skeletal muscle, supporting the theory that skeletal muscle is the primary source of increased production [25]. Epinephrine acts by stimulating muscle and hepatic phosphorylase and inhibiting glycogen synthetase. This results in increased cyclic AMP production and stimulation of Na-K-ATPase and glycogenolysis [25,26]. Na-K-ATPase generates ADP, which accelerates aerobic glycolysis and therefore lactate concentration [25]. These data from human medical studies further supports the notion of lactate generation even in the presence of adequate oxygen, albeit at a much lower rate than in patients will systemic illness. Therefore, the presence of lactate alone is not adequate for proof of systemic or tissue level hypoxia.

1.1.5 Lactate as a Measure of Treatment Response

Lactate measurements have been used clinically to monitor response to treatment in both human and veterinary medical fields. Clinically centered research

on lactate has focused primarily on its ability to be utilized as a mechanism of monitoring goal-directed therapies as well as its ability to predict survival and the risk of development of significant complications. Studies undertaken in human medicine have served as a foundation for veterinary research, and sufficient data have been gathered to provide guidelines for therapeutic goals during the initial phase of treatment in critical cases.

In human patients with sepsis and septic shock, lactate production occurs partially because of oxygen delivery failing to meet the increased tissue oxygen demands. This results in elevated lactate accumulation and eventual acidosis and it is common to monitor lactate clearance as treatment progresses. Research showed that even modest increases (>4 mmol/L) in lactate, if seen conjunction with evidence of the systemic inflammatory response syndrome (SIRS) significantly affects mortality [27,28]. Monitoring serial progression of lactate can allow for prediction of survival and the risk of developing multiple organ dysfunction syndrome (MODS) [29,30]. The half-life of lactate is approximately 20 minutes [31]. Therefore, early clearance of lactate within the first six hours of hospitalization has been associated with improved outcome [28]. Specifically, for every 10% increase in lactate clearance, an 11% decrease in the likelihood of mortality has been demonstrated [28]. Additionally, patients with a greater than five percent clearance in the first hour or greater than 10% clearance in the first 6 hours of therapy had an overall lower 60-day mortality rate compared to those who did not [28].

Another aspect of treatment aimed at improving outcome in human patients with sepsis and septic shock was the “early goal directed therapy” developed in 2001

[32]. This therapy is a comprehensive treatment approach aimed at restoring tissue perfusion and oxygenation through the use of crystalloid fluids, vasopressors, vasodilators and red cell transfusions in the initial phase of treatment [32]. These patients are continually monitored for perfusion parameters such as urine output, blood pressure, vital parameters and central venous pressure [32]. In addition, repeated arterial and venous blood gas measures are performed to assess how well the patient is responding to therapy and include measurements of central venous oxygen saturation ($ScvO_2$) and lactate [32]. Results of this treatment approach compared to the earlier standard approach to patient resuscitation resulted in markedly increased rates of short and long-term patient survival both in the short- and long-term [32]. Measuring $ScvO_2$ requires the placement of a central venous catheter, which requires expertise and time for placement and specialized equipment to complete the measurements [33]. This is a significant impedance for utilization in a clinical setting [33]. Therefore, additional research was performed to assess the non-inferiority of lactate measurement as a goal for early sepsis resuscitation [34]. This study found that lactate measurement as a goal for resuscitation therapy was just as effective as measuring $ScvO_2$, providing a rapid, inexpensive and readily available option for continual blood monitoring in the early resuscitative period [34].

These findings appear to be applicable to veterinary medicine. In equine medicine, lactate has become a commonly used marker of tissue hypoxia and systemic hypoperfusion in a variety of critical illnesses. In adult equine patients with severe illness, hyperlactemia is most commonly related to hypovolemia and shock. With septic equine patients, other factors previously mentioned relating to

hyperlactemia in human medicine, such as catecholamine release, increased aerobic glycolysis and inflammatory responses from leukocytes, also contribute [35,36]. Where equine medicine tends to differ from human medicine is that in many horses, even those with severe clinical disease, lactate tends to normalize in the initial treatment phase even in those that have a poor long term outcome [37]. However, similar to human patients, lactate tended to increase in the 48-72 hours after initiation of treatment in non-survivors, albeit this elevation was quite subtle in some cases sometimes even remaining within the normal reference range [37].

Of special note are the differences between adult and neonatal equine patients. Horses in the neonatal period have a higher normal lactate values than adults. Immediately post-partum venous lactate has been reported up to 4.9 ± 1.0 mmol/L in the healthy foal that decreases to normal within 24 hours after birth [38,39]. This should be taken into consideration when assessing neonatal foals with critical illness, which can commonly present within this time frame.

1.1.6 Lactate as a Prognostic Tool

In addition to lactate being a useful tool for monitoring response to treatment, it also has prognostic value. If lactate does not decrease appropriately, even in the face of appropriate treatment, the prognosis for survival is generally considered worse in both human and equine medicine [3,27,37,40,41]. This allows a potential opportunity for early recognition of an inappropriate therapeutic response, which can then serve as a potential indicator for a need to change therapy or to perform additional diagnostics.

Initial research in human patients suggested that blood lactate on admission was significantly associated with the presence of shock as well as survival [42]. Later research showed that the severity of lactate at admission was of use in determining disease severity and also has prognostic value for mortality, the development of MODS, and other complications [41,43,44]. However, more value was found in the serial monitoring of lactate over time than on the sample at presentation [41,43,44]. While patients may present with moderate to severe elevations in lactate, if their clearance mechanisms are functioning properly or resume function quickly, limited long-term organ damage will occur with early and aggressive treatment [29,32]. In addition, when excluding severely affected patients who die within the first 24 hours of treatment, there is no significant difference in presenting lactate severity between survivors and non-survivors [29]. In human medicine, the best factor recognized for predicting survival and the development of MODS is the duration of hyperlactemia in the face of appropriate therapy and patients who are unable to normalize their lactate values despite treatment approach 100% mortality [29,41]. In children, increased mortality and risk of development of MODS has been demonstrated with persistent hyperlactemia after 12- and 24-hours of treatment and the likelihood of non-survival and development of MODS increasing significantly with the severity of hyperlactemia [30,45].

In equine veterinary medicine, the utility of lactate as a prognostic tool was first explored in the 1970's, where the jugular lactate value in horses presenting to emergency hospitals for colic was determined to have best utility for prognostic as opposed to diagnostic purposes [46]. Research in these horses focused initially on the

lactate value at admission and found clinical utility in determining their need for surgery as well as prognostic value for survival in these cases [46,47]. These findings were then applied to other individuals, with admission hyperlactemia demonstrating prognostic value in neonatal foals presenting for surgical and non-surgical conditions [35,36,48]. However, similar to human medicine, more prognostic values has been shown with monitoring serial lactate values over time [35,37,48].

Of interesting note, recent research has demonstrated a difference in the blood lactate concentrations at presentation in miniature horses and ponies compared to horses [49]. Both of these equid groups were shown to have significantly higher blood lactate concentration at presentation than horses [49]. Additionally, ponies with non-strangulating lesions, large intestinal diseases requiring only medical therapy and surviving ponies had significantly higher lactate values than horses in the same categories [49]. Also, no difference in survival was found in ponies with surgical compared to non-surgical lesions or ponies with strangulating compared to non-strangulating lesions [49]. Therefore, blood lactate on admission does not appear to be as reliable in determining disease severity and likelihood of survival in these groups when using conventionally accepted values and these breed differences should be considered when making treatment decisions and recommendations to clients [49].

In addition to serial measurement of venous lactate, comparison of the venous lactate to lactate in other bodily fluids has demonstrated benefit in equine medicine for diagnosis and prognosis. Commonly, peritoneal or pleural fluid lactate is compared to jugular venous lactate. Under normal circumstances, peritoneal fluid lactate should be equivalent to or slightly less than venous lactate [50]. When the lactate

in the peritoneal fluid starts exceeding that of the jugular vein it has significant implications on abdominal health and the prognosis for survival begins to decrease significantly once the abdominal fluid lactate exceeds 8.5 mmol/L [51]. The prognosis becomes poor to grave once the peritoneal fluid lactate excedes 12 mmol/L[51]. When this information is taken into consideration along with values such as glucose, total protein, pH and leukocyte count and morphology then this can indicate conditions such as gastrointestinal strangulation, peritoneal inflammation, sepsis, or neoplasia [52]. Similar prognostic and diagnostic capabilities have been demonstrated with analysis of pleural, synovial, pericardial, and cerebrospinal fluid [52].

1.2 Vascular Anatomy and Blood Sampling

1.2.1 Basic Vascular Anatomy

Oxygen necessary for the aerobic metabolic processes of cells is supplied to the tissues of the body through the vascular system. The circulatory system is a closed system comprised of large arteries that become progressively smaller and more complex as they move out toward tissue beds, forming a vast capillary bed network at the level of the tissues allowing for distribution of oxygen to the tissues. These arterial capillaries anastomose with venous capillaries that slowly enlarge to form veins that drain deoxygenated blood, lactate and other waste products from the tissues to the liver, lung and heart for continued metabolism, excretion and circulation.

Veins, compared to arteries, typically have thinner walls and are easily collapsible [53]. While the arteries are thick walled and movement of blood is directed under pressure from the beating of the heart, veins are generally maintained under less pressure and rely on the continuous supply of blood from arteries and function of valves along the venous walls to ensure unidirectional flow of blood toward the heart [53]. These valves prevent the back flow of blood during stagnation of certain vessels and are usually found in vessels that are exposed to intermittent changes in external pressure, such as those that are found between muscles and in antigravity veins in the distal limb of the horse [53].

The entire vascular system is under control of the nervous system and it receives both sensory and motor inputs [54]. The majority of vasomotor nerves are found on the arterial side of the circulation, are part of the sympathetic nervous system and have vasoconstrictive properties responsible for generating peripheral resistance to blood flow [54]. This system allows for shunting blood towards or away from different parts of the body depending on the needs of the animal [54]. For example, during exercise or periods of high sympathetic tone, blood is shunted away from the gastrointestinal tract and toward the skeletal musculature in order to maximize oxygenation of these tissues during periods of increased demand [55]. These neural controls are stimulated by a variety of inputs from local and distant electrical, chemical and pressure inputs to alter vascular tone [55].

The veins drain blood from local and regional tissue beds. The paired external jugular veins in the horse are responsible for draining the majority of the blood from the head of the horse, including the highly metabolic tissues such as the brain, as the

internal jugular veins are essentially non-existent [54]. The cephalic vein is responsible for draining all the vascular tissues of the fore limb [54]. Below the level of the carpus in the horse, the largest metabolic tissue in the distal limb are the laminae of the foot, as the remaining structures are largely tendons, ligaments, bones, and connective tissue.

1.2.2 Comparative Blood Sampling in Veterinary Medicine

The most common site for blood collection in horses is the jugular vein [56]. Due to the potential complications, desire to preserve the jugular vein for intravenous catheterization or medical conditions preventing the use of the jugular vein, alternative sites of venipuncture have been investigated including the facial sinus, cephalic vein, saphenous vein and superficial lateral thoracic vein [57]. Each site has utility, but some concerns such as safety, ease of sampling and accessibility prevent them from routine use [56].

The transverse facial venous sinus is most commonly utilized as most horses are tolerant of the procedure and up to 35 ml of blood can be collected [56]. When comparing transverse facial sinus samples to those of the jugular vein, multiple studies found them to be highly correlated, which is not surprising, since both drain the tissues of the head [56,58-60]. The variables that differ, including ionized calcium and glucose are not considered clinically significant [60].

The cephalic vein is another common alternative site of blood collection in the horse that is considered relatively safe to sample and is generally well tolerated.

More limited data has been gathered on the comparison of values obtained from the cephalic vein to the jugular vein. Most studies have investigated the correlation of packed cell volume (PCV) and total solids (TS) between the two sites and have found no clinically significant differences [58]. Recently, several studies have compared jugular to cephalic venous lactate concentrations in healthy dogs and sick cats [61,62]. These studies found small but clinically insignificant differences between sites in dogs and no significant differences in cats [61,62]. However, to the author's knowledge, no such studies have been investigated beyond the comparison of PCV and TS in horses, which serves as the foundation for the impetus of the research subsequently undertaken.

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2. COMPARISON OF CEPHALIC TO JUGULAR VENOUS BLOOD GAS ANALYSIS IN CLINICALLY ILL HORSES

2.1 Introduction

Lactate is a commonly used as an indirect measure of tissue perfusion in human and veterinary medicine. Commonly, lactate is produced when there is insufficient oxygen to support the oxidative phosphorylation pathway of ATP generation, resulting in the excessive accumulation of pyruvate, NADH, and H^+ [1]. However, even though lactate is more commonly produced during anaerobic glycolysis, it can also serve as an end product of the aerobic pathway [2,3]. Therefore, even in normal healthy animals, a basal amount of lactate is constantly being produced and metabolized [2,4]. Increases in lactate production in patients presenting for critical illness can represent either an increase in lactate production, decrease in lactate clearance or a combination of the two. In critical illness, increased lactate production can be related to tissue hypoxia, abnormal cellular metabolism, recruitment of production from other tissues and decreased clearance of lactate due to organ dysfunction [3,5-11].

In veterinary medicine, fluid samples from different body cavities are often sampled and compared to jugular lactate in an effort to identify a source of production, with the abdominal and thoracic cavities being the cavities most frequently sampled. Measurement of abdominal and jugular lactate has been used to help identify horses having intestinal ischemia, are in need of surgical interventions

and to help predict the likelihood of survival [12-15]. Additionally, serial lactate measurements have been utilized in neonatal and adult critical illness in equine veterinary medicine to monitor response to treatment and to predict complications and survival [14,16-21].

An additional method of comparison involves sampling blood from different venous sites. The hypothesis behind this sampling comparison is that changes in markers of tissue metabolism from different regions would be reflective of the different metabolic demands of the regional tissues. When comparing different blood sampling sites, limited studies have been performed in the horse. The most detailed studies have compared sampling of the transverse venous sinus to the jugular vein, with very little difference noted between these two sites [22-24]. From an anatomical perspective, this conclusion would be logical, in that the jugular vein is considered a "downstream" site from the transverse facial sinus, therefore they would share similar venous metabolic profiles. Other unrelated anatomic sites have also been investigated in the horse, but these studies have been limited in the comparison that has been done [25]. Slightly more in-depth analysis has been performed in small animals, with varying results [26,27]. However, to the authors knowledge, no similar study has been performed in horses.

The purpose of this study was to compare jugular to cephalic venous blood gas variables, with a focus on plasma lactate concentrations in both healthy and critically ill horses. The cephalic vein was chosen for two reasons. First, it is easily accessible and commonly used in horses where there is a particular reason to avoid using the jugular vein, such as with concerns about jugular thrombosis. Second, the

cephalic vein drains the fore limb, which is a long, exposed structure with potential for compromised blood flow and terminates in the highly metabolic laminae of the foot. The authors hypothesized: cephalic lactate would be significantly different from jugular lactate in the sick horses, but not in the healthy horses; the severity of the difference in the sick horses at presentation would correlate to the likelihood of the horse developing complications or not surviving to discharge; and sick horses that did not survive or developed complications would also have a poorer response to treatment.

2.2 Materials and Methods

2.2.1 Funding and Approval

Funding for this project was provided through the Oregon State University, College of Veterinary Medicine - Department of Clinical Sciences Resident Research Grant. No conflicts of interest are declared.

The study, and all animal use and sampling protocols were approved by the Oregon State University Institutional Animal Care and Use Committee (IACUC).

2.2.2 Control Subjects

Ten healthy, mature horses from the Oregon State University, College of Veterinary Medicine Teaching Herd were used as the control horse population. Each

horse had a physical examination performed prior to sample collection to ensure that they were free of overt signs of clinical illness and injury. All horses were mares and ranged in age from 10 to 26 years old. Breeds represented included: Thoroughbred (4), Paint (2), Quarter Horse (2), Dutch Warmblood (1), and Belgian Draft (1).

2.2.3 Case Subjects

Twenty-four horses met the criteria for inclusion in the study, which included any horse presented to the Oregon State University, College of Veterinary Medicine for treatment of suspected enterocolitis. These included horses that were admitted for an acute onset of colic-like behavior, with one or more of the following additional criteria of a fever, diarrhea, or a low total white cell count. These horses ranged in age from 2 to 30 years old. Breeds represented included: Quarter Horse (11), Paint (3), Tennessee Walking Horse (2), Warmblood (2), Appaloosa (1), Arabian (1), Haflinger (1), Mustang (1), Pony of the Americas (1), and Cross Breed (1). Eight of the horses were mares and sixteen were geldings.

2.2.4 Sample Collection

For control horses, each horse was walked for approximately 30 steps prior to sample collection to mimic the distance walked by case horses from their trailer to the isolation stall. After walking, a blood sample was collected from the cephalic vein on the medial aspect of either proximal forelimb proximal to the carpus (Figure 2). Once

the cephalic sample was collected, a jugular blood sample was collected. Samples were collected into heparinized 3 ml syringes with a 20-gauge needle. Samples were evacuated of any air, to prevent oxygenation and immediately processed on the in-hospital blood gas analyzer[1]. A single paired jugular and cephalic sample was collected for each control horse.

For clinical cases, each horse was sampled in an identical fashion to the control horses. Initial samples were collected on admission (time 0). Additional cephalic samples were also collected any time during the first 24 hours of hospitalization when a jugular venous blood gas sample was done as part of the clinical management of the case under the direction of the supervising clinician. A maximum of 3 cephalic samples were collected from any horse during the initial 24-hour period of hospitalization. Additional data were then collected retrospectively from the medical record including: days of hospitalization, survival, if ice boots were used, if complications developed (including laminitis) clinical diagnosis, and findings on necropsy, if performed.

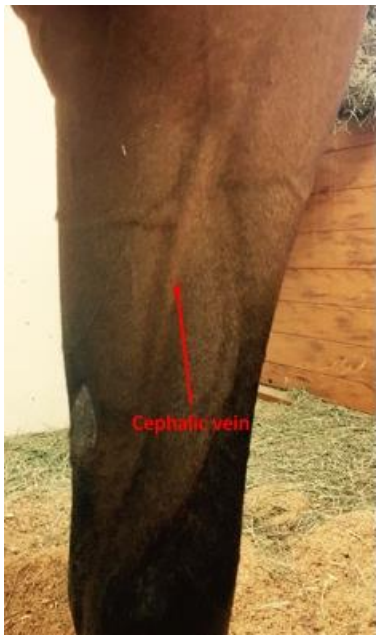

Figure 2: Site of Cephalic Sample Collection

[1] STAT Profile® pHOx® Ultra™. Nova Biomedical: Waltham, Massachusetts

2.2.5 Statistical Analysis

All data was analyzed using commercially available statistical software[2]. Statistical significance was determined to be a result with a p-value less than or equal to 0.05. For data that had multiple variables to consider, mixed effects modelling was performed.

2.3 Results

2.3.1 Descriptive Data Results

Table 2.1 summarizes the population of horses that were included in the case group. A total of 24 cases met the inclusion criteria of the study. Of the 24 horses, 7 (29.2%) did not survive hospitalization (sick, non-survivors), 10 (41.7%) developed some type of complication during hospitalization, and 4 (16.7%) developed acute laminitis (sick, laminitis) that was either noted clinically or during post-mortem examination. Only one of the four (25%) horses with laminitis survived to hospital discharge.

The average duration of hospitalization for all sick horses was 7.5 days. For sick survivors, average duration of hospitalization was 8.9 days. For sick non-survivors, the average duration of hospitalization was 4.2 days.

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[2] R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Table 2.1: Summary Information for Horses Included in the Sick Group

Animal Number	Age (years)	Gender	Breed	Diagnosis	Complications	Survive Y/N	Days in Hospital
1	9	F	Halflinger	Colitis - unknown	--	Yes	5
2	8	G	Quarter Horse	Colitis - unknown	Laminitis, DIC,	No	7
3	11	G	Paint	PHF	--	Yes	6
4	5	G	Warmblood	Colitis - unknown	--	Yes	11
5	23	G	Quarter Horse	Colitis /peritonitis	--	Yes	8
6	8	F	Quarter Horse	Colitis - unknown	Thrombophlebitis	Yes	16
7	19	G	Swedish WB	Lymphoma	--	No	1
8	16	G	Quarter Horse	colitis - unknown	Pleuropneumonia/Laminitis	No	7
9	19	F	TWH	Colitis - unknown	--	Yes	8
10	13	F	POA	typhlitis	Abdominal abscess	Yes	15
11	19	G	Mustang	PHF	--	Yes	6
12	12	G	Mixed Breed	Colitis - unknown	--	Yes	4
13	8	G	Quarter Horse	PHF	--	Yes	5
14	7	G	Quarter Horse	PHF	DIC	No	2.5
15	5	F	Quarter Horse	Coronavirus	Catheter site swelling	Yes	7.5
16	4	F	Arabian	Colitis - unknown	Catheter site swelling	Yes	4
17	8	G	TWH	Colitis - unknown	--	No	10
18	30	G	Quarter Horse	Suspect lymphoma	--	Yes	22
19	23	G	Paint	PHF	Laminitis	No	1
20	5	G	Quarter Horse	PHF	Laminitis	Yes	7
21	17	F	Paint	PHF	--	Yes	9
22	2	G	Quarter Horse	Colitis - unknown	--	Yes	12
23	18	G	Quarter Horse	Colitis - unknown	--	Yes	6
24	10	F	Appaloosa	Colitis - unknown	Laminitis	No	1

2.3.2 Effect of Sample Site on Lactate

The first hypothesis investigated if there was a difference in the lactate values between the jugular vein and the cephalic vein. This was investigated for the control horses and clinical case horses. Additional comparison of sites was also performed on

sub-groups of horses within the clinically affected horse population for horses with and without laminitis and horses that survived and non-survivors. A one-sample t-test on paired data was used for each group. For the clinically affected horses and all sub-groups investigated, the sample collected at presentation was used for analysis. A statistically significant difference was found in all groups, including the control horse population, except for the sub-groups that included clinical cases that were non-survivors (p=0.1087) and clinical cases that developed laminitis (p=0.1212) (Table 2.2). This lack of significant difference was attributed to the small sample size in these groups with those outcomes since only seven horses were non-survivors and four horses developed laminitis.

Table 2.2: Mean Jugular and Cephalic Lactate Values and Associated P-Values

Group	Mean Jugular Lactate (mmol/L)	Mean Cephalic Lactate (mmol/L)	P-Value
Healthy Control*	0.7	1.23	0.000714
All Sick Horses*	3.2	3.74	$3.34x10^{-10}$
Sick – Non-Survivors	4.1	4.73	0.1087
Sick – Survivors*	2.7	3.33	0.0001038
Sick – Non-Laminitis*	3.3	3.85	0.0005082
Sick – Laminitis	2.5	3.20	0.1212

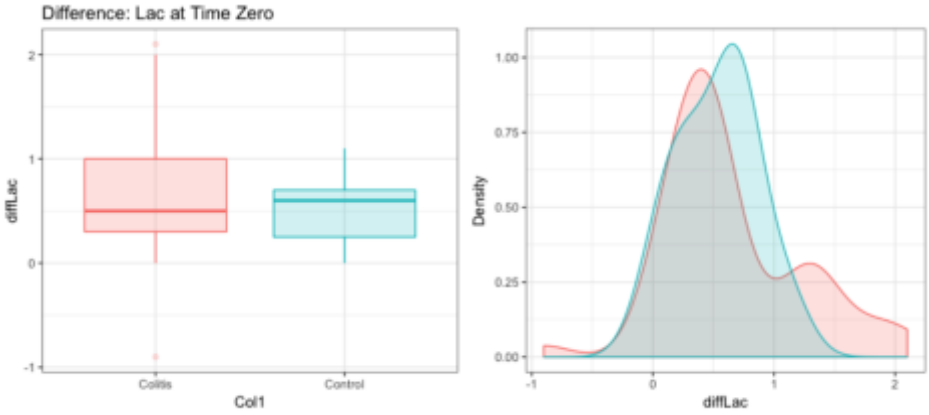
* Indicated a statistically significant difference in mean values between sites.

2.3.3 Effect of Clinical Disease on Lactate Difference

The second hypothesis investigated if there was a statistically significant difference in the lactate of the jugular vein compared to the cephalic vein in horses presenting for colitis compared to the healthy controls. A two sample, t-test on paired

data was used to compare the difference in lactate values between sampling sites at presentation in healthy and systemically ill horses. At presentation, there was no difference (p=0.403) between healthy horses and those presenting with colitis when comparing the difference in their lactate values between the jugular and cephalic veins (Figure 3).

Figure 3: Difference at Presentation in Juglar and Cephalic Vein Lactate in Healthy and Clinical Cases



The difference in lactate values between the two venous sites at presentation was then compared across outcomes, assessing both survival and the development of laminitis. For each comparison, a two-sample t-test on paired data was used to compare the two groups (survivors vs. non-survivors and laminitis vs. non-laminitis). Neither group demonstrated a significant difference. When comparing survivors to non-survivors the p-value was 0.095 (Figure 4) and when comparing horses that developed laminitis to those that did not, the p-value was 0.11 (Figure 5). This means the degree of difference in lactate between sites at presentation does not appear to

demonstrate an ability to predict horses that are more likely to develop laminitis or not survive hospitalization.

Figure 4: Difference at Presentation in Juglar and Cephalic Vein Lactate in Survivors and Non-Survivors

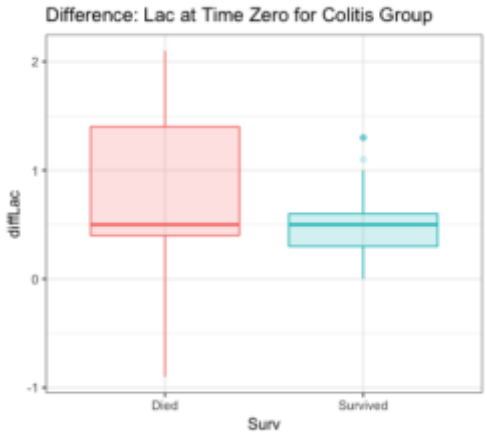
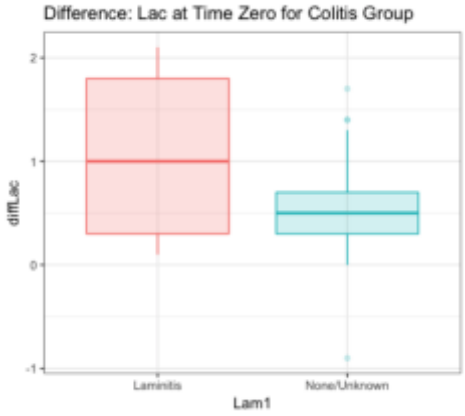


Figure 5: Difference at Presentation in Juglar and Cephalic Vein Lactate in Horses with Laminitis and Without Laminitis



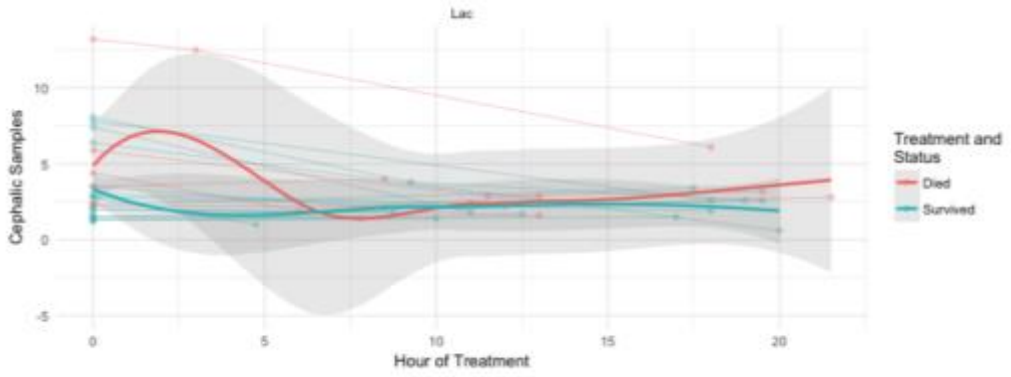
2.3.4 Effect of Time on Lactate

The final hypothesis investigated looked at the change in lactate difference between sample sites over time. This looked at the change in difference in lactate through time to determine if there was a difference in horses that developed laminitis or were non-survivors. Additionally, a linear mixed model was also performed to assess the cephalic lactate values through time for each outcome (survivor vs. non-survivor and laminitis vs. non-laminitis). When comparing horses that survived to those that did not survive, the model showed that there was no difference in the cephalic lactate between groups ($p=0.97$) (Figure 6). This means that there was no difference in the rate of change in cephalic lactate over time for survivors compared to non-survivors. Jugular lactate was not assessed with the model but was assumed to follow a similar path.

The linear mixed effects model was then repeated looking at the difference in lactate between sites in sick horses and for each of the outcomes (survivor vs. non-survivor and laminitis vs. non-laminitis). When comparing horses that survived to those that did not survive, the model showed that there was no difference in the rate of change of the lactate difference between sites over the first 24 hours of hospitalization ($p=0.1551$). When comparing horses that developed laminitis to those that did not, the model again showed no difference in the rate of change of lactate difference between sites over the first 24 hours of hospitalization ($p=0.4285$). This means that both models showed a similar decreasing trend in lactate and given the standard deviation that showed significant overlap (Figure 6) this also means that

there was not a significant difference between groups at any time point. However, it is important to note that only four horses developed laminitis and therefore the strength of the model was considered very weak.

Figure 6: Lactate Levels in Cephalic Samples Over Time in Systemically Ill Horses (Survivors vs. Non-Survivors)



2.4 Discussion

2.4.1 Conclusions

Previous studies in horses that compared different site of blood sampling to the jugular have not identified significant differences [22-25]. However, when looking at the cephalic vein compared to the jugular vein very limited studies has been done and has not expanded beyond the comparison of packed cell volume and total protein [25]. To the author's knowledge, this is the first study comparing lactate measures between these sites in horses. In most of our sample groups, including our healthy control

population, the lactate measure in the cephalic vein was significantly higher than in the jugular vein. This suggests a higher rate of glycolysis in the leg than the head, or a lower rate of lactate utilization. The only groups this was not appreciated was in the sick, non-surviving population and the sick, laminitis population, suggesting in those horses that systemic under perfusion was overshadowing the subtle changes happening in the leg. Unfortunately, this has significant implications for the remaining hypotheses of the study, because the subsequent null hypothesis was contingent on the lactate values between sites in healthy horses being equivalent. However, it is important to note that while there was a statistically significant difference between these sites in healthy horses, in all the control horses the value at both sites remained within the normal reference range (<2 mmol/L).

These findings suggest that clinicians should be cautious in using cephalic and jugular values interchangeable. This is particularly important when the blood value is being compared to a sample from another body compartment, such as peritoneal fluid, or when serial samples are being taken to monitor progress. The 0.6 to 0.7 mmol/L lactate differences between the two sites are large enough to potentially affect the interpretations in those comparisons.

These findings also imply that there is a smaller difference in the lactate measured between sites at presentation in horses with poorer disease outcomes (i.e. those that will not survive or will develop laminitis) compared to healthy horses and those with better outcomes. There are several possibilities as to why this may occur. One possible reason is that these horses may have a more severe degree of hypoperfusion. This hypoperfusion could result in an increased amount of systemic

anaerobic metabolism and lactate production compared to other groups or a decreased rate of lactate clearance from the distal limb, resulting in a narrower difference between the lactate values in the jugular and cephalic veins. These cases may also be more compromised than their counterparts and therefore have increased lactate production systemically due to sepsis, endotoxemia or increased systemic inflammatory responses, which could decrease the difference in lactate between these two sites since the cephalic sample was always the higher value. Additionally, in horses with laminitis arterio-venous shunts in the distal limb become patent and blood flow to the foot can be bypassed, at least in part. Therefore, this lack in change in even the more severely affected horses or those that developed laminitis may reflect this shunting.

The second hypothesis investigated the comparison of lactate difference in healthy and clinical cases. The authors hypothesized that diseased horses would have a significantly greater difference in the lactate values between sites than the difference seen in heathy horses. The data collected in this project failed to reject this hypothesis, which indicates that overall, clinically ill horses in this study did not have a larger difference in lactate than their healthy counterparts. This is likely a complication from rejection of the first null hypothesis that demonstrated a significant difference between sites in healthy horses as well as clinical cases. Additionally, no significant difference was found when comparing these differences in the sub-groups of clinical cases (laminitis vs. non-laminitis and survivors vs. non-survivors). This is not surprising given previous research that has demonstrated limited prognostic value from data obtained at hospital admission. Further research investigating lactate

kinetics in healthy horses is warranted to better understand the significance of the differences between sites.

The third hypothesis investigated the change in lactate over time with the ability to determine outcome (survival vs. non-survival and laminitis vs. non-laminitis) in clinical cases. This was performed with mixed effects modeling and looked at both total cephalic lactate values as well as the difference in lactate values. For total cephalic lactate, changes in cephalic lactate over time did not demonstrate a significant difference when comparing survivors to non-survivors or comparing horses that developed laminitis to those that did not. Both groups showed a steady decrease in lactate over time in response to therapy. When looking at the confidence intervals for these individual groups, as demonstrated by the grey shaded areas in Figure 6, there is significant overlap at all time points for each group that was compared. Together, this means that at least within the first 24-hours of treatment, lactate in both sampling sites decreased and that the rate and severity of decrease does not appear to be significantly different between any group, regardless of outcome. These findings are consistent with other research, that have demonstrated that many horses, even those that do not do well, will show an initial decrease in lactate in response to treatment and may even be within the normal reference range [14,20]. Additional benefit can be gained through serial measurements to monitor response to treatment, as overall lactate trends, even when they are within the normal reference range, can provide quality information in identifying animals and people at higher risk of complications and death [3,14,28].

2.4.2 Study Limitations

One of the major limitations of this study was the inconsistency in repeated samples. Because a statistically significant difference in lactate values between sites was found in the control horses, repeated samples performed over the same time period as the clinical cases should have been performed to see how these values vary over time. Additionally, clinical cases should have had an increased number of and more consistent repeated samples taken after admission, to better track changes in response to treatment and to make comparisons across the group as a whole. Ideally, blood samples would have been collected every 2-3 hours throughout the first 24-72 hours of treatment to allow for more consistent tracking of blood lactate values. Due to the infrequency of repeated samples, more refined analysis through time was not possible. The use of a cephalic catheter may potentially limit complications related to repeated sampling of the affected limb. Similar to other clinically based studies, a larger sample size of clinical cases and use of a multi-center study would have increased the power of the information obtained and been a more accurate representation of the equine population.

Another limitation of the study was the limited number of horses that developed laminitis. One of the main objectives of the study was to determine if cephalic blood gas analysis had any value in determining survival, but also in determining if these horses were more likely to develop complications with a specific focus on laminitis. With only four of the 24 horses developing laminitis, this

significantly limits the data analysis in determining any significant values for utilization. The number was too low for some of the mathematical models to accurately perform statistical analysis on the lactate measurements obtained, which limited our ability to evaluate the relationship between lactate and laminitis development beyond the samples obtained at admission.

Lastly, the finding of statistically significant differences in lactate values between sites in clinically healthy horses was an unexpected outcome. Based on these results, a more in-depth preliminary investigation should be performed in healthy horses to better understand the lactate kinetics of the cephalic vein and associated fore limb and how much variation can be considered normal. Better understanding of the healthy horse population will allow for better interpretation of the data obtained from the clinical cases. Future studies should first be aimed at investigating a larger group of healthy horses and repeated samples should be obtained over time to try and establish how these values change over time.

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3. CHANGES IN LACTATE IN HORSES UNDERGOING PROLONGED STANDING SEDATION

3.1 Introduction

Alpha-2 adrenergic agonists including xylazine, romifidine, detomidine, and met-detomidine are commonly used sedatives, analgesics and anesthetic pre-medications in veterinary medicine. In equine medicine, detomidine is commonly used for standing procedures as a means of standing sedation and chemical restraint [1]. Activation of α-2 adrenergic receptors has a variety of effects depending on the location and the type of the receptor. If the pre-synaptic receptor is activated there is inhibition of the release of the catecholamines, norepinephrine and epinephrine, resulting in vasodilation and slowing gastrointestinal motility [1]. If the post-synaptic receptor is activated sedation, analgesia, decreased anxiety, inhibition of insulin release and vasoconstriction is appreciated [1]. The inhibition of insulin results in a transient but significant hyperglycemia that is reversible with the administration of α-2 antagonists [2].

There are three different sub-types of α-2 receptors (α-2A, -2B, and -2C), however detomidine is considered a non-discriminatory agonist and will bind to all three subtypes and these receptors are responsible for different responses [1]. Alpha-2A receptors largely are responsible for sedation, inhibition of insulin secretion, neuroprotection and sympathetic responses [1,3]. Alpha-2B receptors mediate spinal analgesia and vasoconstriction and α-2C receptors play a role in pain modulation,

locomotor activity suppression and catecholamine release from the adrenal medulla [1,3]. Therefore, with detomidine binding non-discriminately to all three receptor sub-types a wide range of effects can be seen.

Detomidine is used frequently in horses for the purposes of standing sedation and when provided as a single intravenous dose it has a half-life of approximately 30 minutes [4]. This half-life can be extended to 60 minutes, albeit with a lower peak plasma concentration, if administered intramuscularly [4]. Therefore, when medium duration (20-40 minutes) sedation is required in clinically stable horses, intravenous detomidine is well suited for this purpose. If more prolonged sedation is required, this can be achieved through repeated bolus administration or by using a continuous infusion. The continuous infusion is considered superior to intermittent bolus techniques for prolonged sedation (such as for standing surgical procedures), due to the steady plane of sedation and analgesia achieved [1,2,5]. This method limits movement of the patient during procedures and provides the clinician with the ability to rapidly increase or decrease the plane of sedation by changing the rate of the intravenous infusion without the need of administering reversal agents[1,2,5].

Long term sedation with a non-selective α-2 agonist, such as detomidine, can lead to side effects due to their non-discriminatory binding. Previous studies have documented significant decreases in heart rate, respiratory rates and circulating catecholamine and plasma insulin concentrations and increases in circulating glucose and lactate parameters [2,4,6,7]. While these changes were statistically significant, not all changes were clinically relevant. For example, frequently the lactate values, heart rate and respiratory rates were reported to be significantly different from their

baseline values but they were still within the normal reference range for horses and therefore not considered clinically significant [2,7]. To the author's knowledge, the effects of long term sedation with a continuous infusion of detomidine beyond 60 minutes on lactate, hear and respiratory rate are unknown [7]. Commonly, standing surgical procedures require more than 60 minutes considering sterile site preparation, surgical procedure and site closure. Therefore, the purpose of the study was to examine the effects of long-term standing sedation in healthy, adult horses. We hypothesized that with prolonged sedation a continual increase in lactate and glucose would measure above normal reference ranges. However, other measures of sedation, such as heart rate, respiratory rate and blood pressure would not be significantly altered with increased time.

3.2 Materials and Methods

3.2.1 Funding and Approval

All funding was received through Oregon State University, College of Veterinary Medicine Start-Up Funds through Dr. John W. Schlipf and through the Oregon State University, Department of Clinical Sciences Resident Research Grants. No conflicts of interest are declared. The study and all protocols used were approved by the Oregon State University Institutional Animal Care and Use Committee (IACUC) ACUP 4774.

3.2.2 Subjects

Eight healthy, mature, full-sized horses from the Oregon State University, College of Veterinary Medicine teaching herd were selected for use in this study. Horses ranged in age from 10 to 21 years old. Three were geldings and the remaining five were mares. The breeds represented comprised of: Quarter Horse (3), Paint (2), Mustang (1), Thoroughbred (1), and Mixed Breed (1).

3.2.3 Study Protocol

Prior to initiating the sedation protocol, each horse had a physical examination to ensure they were free of any signs of illness or systemic disease. A 16-gauge intravenous catheter was then aseptically placed into each jugular vein and sutured in place. Following placement of the catheters, a tail cuff was placed around the base of the tail and a non-invasive blood pressure (NIBP)[3] measurement was taken. Three milliliters of waste blood was removed from the catheter, the initial (T0) blood sample was collected, the catheter was flushed, and a loading dose (15 μg/kg) of detomidine was administered through the catheter. The horse was then placed into the stocks for restraint and started on a detomidine continuous rate infusion at a rate of 5.0 mg/hour for the first 60 minutes of the infusion. The dose was then increased after the first 60 minutes to 6 mg/hour for the remaining 3 hours of the infusion. Blood was collected in the same manner as the first sample from the intravenous catheter

[3] Cardell® 9401 Blood Pressure Monitor. Midmark Coorporation: Versailles, Ohio.

that was not connected to the infusion pump after the first hour (T60) and then every 30 minutes thereafter for full blood gas analysis and lactate measurements (T90, 120, 150, 180, 210, and 240). Lactate measurements were performed on the available hospital blood gas machine[4] as well as on a handheld lactate meter[5] that has been previously validated for use in horses.

Throughout the infusion, the horses were constantly monitored for signs of excessive sedation so the infusion could be decreased, if needed. A complete physical examination (temperature, heart rate, respiratory rate, mucous membrane color and capillary refill time, abdominal and thoracic auscultation and digital pulse palpation) was done every 15 minutes throughout the infusion with NIBP measurements taken at the same time. A final physical examination and NIBP measurement was obtained 15 minutes following cessation of the infusion (T255) to ensure the sedation was decreasing and the animals were becoming more alert.

After the continuous infusion was finished, the intravenous catheters were removed and the horses remained in the stocks until they were alert enough to safely walk back to their stalls. All horses were monitored closely overnight for complications related to the procedure or their intravenous catheters. If no complications were noted, they returned to their regular turn out and feeding regimen the following day. The pre-samples collected at time 0 served as internal controls, since they were collected prior to any drug administration.

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[4] Element POC® Blood Gas & Electrolyte Analyzer. Heska (Cuattro) Corporation: Loveland, Colorado.

[5] Lactate Scout+ Lactate Analyzer®. EKF Diagnostics: Leipzig, Germany

3.2.4 Statistical Analysis

Values including physical examination parameters, lactate, glucose, venous oxygen, carbon dioxide and oxygen saturation were recorded into Microsoft Excel for analysis using both a one sample t-test on paired data. Maximum and minimum values and their difference for each variable were recorded and the means for each parameter were calculated in Microsoft Excel. The threshold for statistical significance was set for each variable at $p \leq 0.05$.

3.3 Results

3.3.1 Sedation Protocol

All horses tolerated the procedure well. No horse was deemed to be excessively sedated during the procedure and no complications were noted following the conclusion of the study. Based on behavioral characteristics all horses were considered to be less sedated than anticipated, especially during the second half of the continuous infusion.

3.3.2 Lactate

Lactate increased from baseline in every horse (Table 3.1). However, at no time point in any horse did the value increase above the upper limit of the normal

reference range. Based on both a one- and two-tailed paired t-test, the increase in lactate was statistically significant ($p=0.05$). The average lactate at the initiation of the study (T0) was 0.94 mmol/L and the average maximum lactate achieved throughout the infusion was 1.3 mmol/L with an average increase of 0.3 mmol/L for each horse. While these values are significantly different, the clinical relevance of these values and changes is likely negligible because all values remained within the normal reference range of <2.0 mmol/L.

Table 3.1: Lactate Minimum and Maximum Values

Horse Number	Lactate (min) (mmol/L)	Lactate (max) (mmol/L)	Difference (mmol/L)
1	0.80	1.9	1.1
2	1.00	1.1	0.1
3	1.20	1.6	0.4
4	0.90	1.1	0.2
5	0.90	1.2	0.3
6	0.80	1.0	0.2
7	1.00	1.0	0.0
8	0.90	1.1	0.2

3.3.3 Other Variables

Other blood gas variables investigated in the study included venous oxygenation measures such as partial pressure of venous oxygen and carbon dioxide, venous oxygen saturation and blood glucose measurements. All these values were significantly different over time but the only value that was clinically significant was glucose ($p<0.05$). The average minimum partial pressure of oxygen was 32.3 mmHg, the average maximum was 41.5 mmHg, with an average change of approximately 9.2

mmHg for each subject (Table 3.2). The minimum oxygen value usually occurred at T0 and the maximal values usually occurred around the mid-point of the infusion. For the partial pressure of carbon dioxide, the average minimum was 47.0 mmHg, the average maximum was 55.6 mmHg, with an average difference of 8.6 mmHg for each subject (Table 3.3). Similar to oxygen, the minimum value was typically at T0 and the maximum value typically occurred at the end of the infusion. The oxygen saturation had an average minimum of 60.5%, maximum of 76.7%, and average difference of 16.2% (Table 3.4). For glucose, the average minimum was 87.6 mg/dL, the average maximum was 244.1 mg/dL, with an average difference of 156.5 mg/dL (Figure 7). This demonstrates an almost 3-fold increase in blood glucose over the course of the infusion (Table 3.5) and all values were above renal threshold by 90 minutes (the as demonstrated by the horizontal dashed line in Figure 7).

For physical exam data, the mean temperature, heart rate, and pulse rate all decreased but remained within normal limits (Table 3.6). The average systolic, and mean arterial pressures for all 8 horses decreased significantly when compared to the values obtained at Time 0 (baseline) ($p<0.05$). The average diastolic pressure also decreased, but was not considered significant ($p=0.064$) (Table 3.6).

Table 3.2: Changes in Partial Pressure of Oxygen

Horse Number	Oxygen (Min) (mmHg)	Oxygen (Max) (mmHg)	Difference (mmHg)
1	42.2	49.4	7.2
2	29.6	34.7	5.1
3	33.3	41.5	8.2
4	38.5	45.9	7.4
5	23.4	36.6	13.2
6	35.1	45.7	10.6
7	26.7	39.2	12.5
8	29.3	38.8	9.5

Table 3.3: Changes in Partial Pressure of Carbon Dioxide

Horse Number	CO_2 (min) (mmHg)	CO_2 (Max) (mmHg)	Difference (mmHg)
1	50.4	60.7	10.3
2	46.1	57.7	11.6
3	42.1	52.7	10.6
4	48.2	54.8	6.6
5	47.7	51.8	4.1
6	48.2	55.9	7.7
7	45.8	55.6	9.8
8	47.2	55.5	8.3

Table 3.4: Changes in Venous Oxygen Saturation

Horse Number	O2 Saturation (Min) (%)	O2 Saturation (Max) (%)	Difference (%)
1	77.1	83.9	6.8
2	57.8	69.4	11.6
3	67.4	79.5	12.1
4	72.0	80.6	8.6
5	40.3	69.4	29.1
6	65.8	82.3	16.5
7	48.6	74	25.4
8	55.0	74.1	19.1

Table 3.5: Changes in Blood Glucose

Horse Number	Glucose (min) (mg/dL)	Glucose (max) (mg/dL)	Difference (mg/dL)
1	91	291	200
2	80	178	98
3	69	322	253
4	88	253	165
5	90	272	182
6	101	225	124
7	91	205	114
8	91	207	116

Figure 7: Glucose Changes Over Time

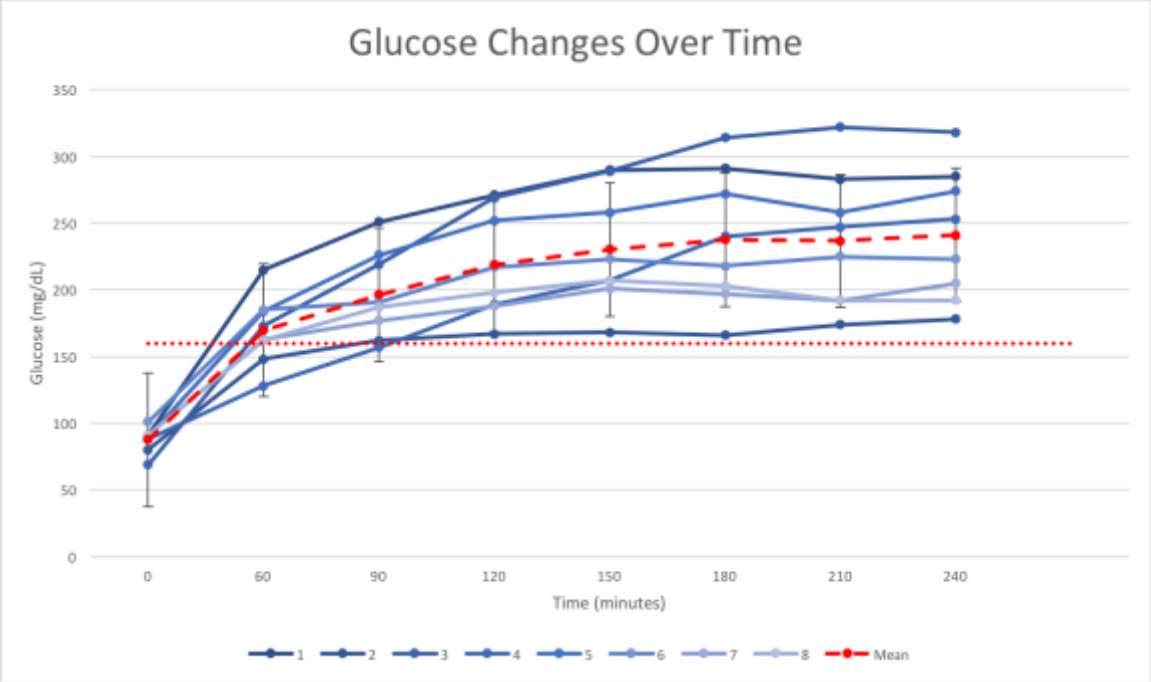

Table 3.6: Physical Exam Summary Data

Horse Number	Mean Temp (F)	Mean Pulse (/min)	Mean Respiratory Rate (/min)	T0 Systolic Pressure (mmHg)	T0 Diastolic Pressure (mmHg)	T0 MAP (mmHg)	Mean Sedated Systolic Pressure (mmHg)	Mean Sedated Diastolic Pressure (mmHg)	Mean Sedated MAP (mmHg)
1	97.4	36.0	12.5	135	121	129	86.71	42.71	56.71
2	97.0	28.5	12.0	137	119	123	86.14	68.57	74.29
3	98.5	36.5	12.0	130	106	113	97.14	61.29	73.14
4	97.8	36.0	16.5	122	79	94	118.43	76.57	91.43
5	97.6	25.5	11.5	101	81	89	86.71	72.00	77.57
6	96.8	25.5	13.0	103	77	84	86.71	55.29	63.86
7	99.6	31.0	11.0	167	92	121	86.71	88.14	98.86
8	99.1	31.5	10.5	95	60	63	86.71	76.43	81.00

3.4 Discussion

Alpha-2 adrenergic agonists such as xylazine and detomidine have previously demonstrated significant cardiovascular effects in horses [8-10]. Detomidine is considered to be approximately 10 times more potent than xylazine and has a greater specificity for α-2 receptors with a longer duration of effects [9]. Following administration of these drugs a decreased heart rate, increased incidence of second-degree atrioventricular (AV) block, reduced cardiac output and transient hypertension followed by prolonged hypotension are commonly seen [8,11,12]. Both of these medications are non-specific α-2 adrenergic agonists and stimulation of these receptors can induce a wide variety of effects including sedation, analgesia, decreased anxiety, hypothermia and cardiovascular effects as discussed above [1,9].

Additionally, administration of α-2 agonists in horses result in hyperglycemia [1,13,14]. This phenomena is attributed to decreased secretion of insulin from the pancreas [2,14]. Sedatives such as detomidine and xylazine act on the β-cells of the Islets of Langerhans which results in hypoinsulinemia that causes the hyperglycemia

[2,14]. In surgical patients the stress response that occurs from surgery may compound the hyperglycemia seen due to the production of hormones, such as cortisol, which have anti-insulin effects [2]. However, previous research has failed to demonstrate this [2].

Previous studies have demonstrated the cardiovascular effects and lactate trends of detomidine used as a continuous infusion over a short period of time for standing sedation [7]. Detomidine infusions have a wide range of dosages with a maximum dose of 36 μg/kg/hr being described [1,5]. Based on these previous studies, we selected a maximum dose of continuous infusion to be set at 6 mg/hr, which was approximately 33% of the maximum reported dose. This rate was selected because it was in accordance with the existing protocol used by the Oregon State University Large Animal Surgery service for clinical cases and was within the published range of effective doses in the horse [1]. The lower infusion rate was selected due to the lack of stimulation these horses would receive while under sedation. Unfortunately, while the initial sedation for the first hour appeared to be sufficient, as the infusion time progressed through the remaining three hours, all horses became progressively less sedated and subjectively did not appear to be in a significant plane of sedation to attempt a standing surgical procedure safely. However, this was the maximum infusion rate that was approved by the Oregon State University IACUC, therefore increasing adjustments to the rate were not possible. In a similar study that examined detomidine infusions over 60 minutes the infusion rate was approximately 20 μg/kg/hr, which was described to achieve levels of sedation that were more similar to clinical settings and may have been a more appropriate dose in this study [7].

The lactate values increased significantly during the infusion, however they never exceeded the normal reference range of 2.0 mmol/L. Lactate is an indirect measure of tissue perfusion and metabolism, as it is a byproduct of increased tissue aerobic and anaerobic metabolic pathways [15-17]. Lactate production is increased from basal levels during periods of decreased tissue hypoxia and during increased catecholamine release [16,17]. The increased lactate in this study was thought to be related to impairment of glucose update in the skeletal muscle due to decreased insulin as well as to decreased skeletal muscle perfusion secondary to peripheral vascular effects of detomidine, resulting in decreased oxygen utilization at the level of these tissues. Previous research has demonstrated detomidine administration can significantly decrease cardiac output and muscle perfusion resulting in increased lactate production by these tissues [10]. Our findings may have also been potentially worsened by hyperglycemia, which was evident in all subjects, which is a reflection of anti-insulin activity but also can worsen hypoperfusion by potentially acting as an osmotic diuretic.

In this study, the circulating glucose very rapidly exceeded the renal threshold of glucose reabsorption (~160 mg/dL) in the horse [6,14]. Detomidine can also cause significant renal sodium excretion, which will contribute to diuresis with or without glycosuria [6]. This enhanced diuresis through either glycosuria or enhanced sodium excretion can contribute to a lower plasma circulating volume. Lower diastolic volume may contribute to decreased tissue perfusion and anaerobic metabolism lactate production and decreased clearance mechanisms.

We report here significant changes in venous oxygen, carbon dioxide and oxygen saturation but do not consider them to be clinically significant. Venous blood gas variables provide evidence of tissue metabolism as opposed to arterial blood gas variables that serve as indices of respiratory gas exchange and blood oxygenation. Overall, the variables identified in this study showed a transient decrease in venous oxygen initially followed by an overall increase. There was an increased partial pressure of carbon dioxide when compared to baseline values. This suggests an initial increase in metabolic activity of these tissues causing an initial increase in aerobic metabolism, resulting in a decreased venous partial pressure of oxygen and increased partial pressure of carbon dioxide.

One of the major limitations of this study was the lack of appropriate sedation. Every horse achieved an immediate plane of sedation that was considered adequate for the first 45-60 minutes of the infusion, however for the remainder of the infusion the horses became progressively less sedate. This was manifested primarily through behavioral signs, such as elevation of the head and responsiveness to stimuli such as touch, noise and motion either within or just beyond the examination room. Subjectively, none of the eight horses by the end of the study period were considered sedate enough to perform standing surgery, meaning this sedation protocol did not adequately mimic clinical conditions. This lighter plane of sedation may have resulted in slower rate of tissue metabolism compared to higher doses potentially due to a smaller induction of catecholamines from the adrenal medulla and improved tissue perfusion due to less severe cardiovascular effects. Additionally, many standing continuous detomidine infusions for surgical procedures combines

detomidine with an opioid such as butorphanol. Future research should be aimed at performing this study either under more clinically relevant levels of sedation, or potentially sampling from clinical cases.

An additional limitation of this study was the inconsistency of the NIBP measurements and the use of the Element POC blood gas analyzer. Non-invasive blood pressure measurements were obtained every 15 minutes throughout the study period. However, these readings were significantly inconsistent with wide range of reported measurements within the same measurement timeframe (repeated measures) as well as on subsequent measures within the same horse. The machine frequently gave error messages that also further undermined the reliability of the measurements obtained. Therefore, assessment of sedation and the circulatory effects of the detomidine was based primarily on behavioral and physical examination parameters throughout the study, as assessment of blood pressure was considered inaccurate. The Element POC was used in this study due to unavailability of the routine hospital bench-top blood gas analyzer. This machine has been validated for use in horses, but is not as reliable as other bench-top machines [18]. To mitigate variability in lactate data, lactate from the same blood sample was also measured on a handheld lactate analyzer that has been previously validated in horses and those values were used for statistical analysis [19].

In conclusion, the utilization of long-term continuous infusion of detomidine appears to have a limited clinical effect on the production and elimination of lactate. However, significant increases in plasma glucose can be seen and in surgical cases hyperglycemia may contribute to increased urine production and subsequent

hypoperfusion, even though this was not demonstrated specifically in this study. In the author's clinical experience, significant accumulations of lactate have been seen in healthy horses undergoing procedures requiring prolonged standing sedation, but we were unable to experimentally re-create this phenomenon here. Future studies with horses undergoing surgical procedures or using infusion protocols that better mimic a clinical setting are warranted to further study lactate accumulation and metabolism.

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4 CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Summary

Lactate serves primarily as an end product of anaerobic cellular metabolism of glucose. However, a degree of production occurs in aerobic environments as well, commonly from the circulating erythrocytes that do not contain mitochondria and are incapable of oxidative phosphorylation and from the small amount that escapes other cells capable of oxidative phosphorylation. Increases in lactate can result from a variety of conditions and commonly reflect a degree of tissue hypoxia, dysoxia, or increased cellular metabolism. Lactate is metabolized by the body primarily in the liver to re-form glucose or is broken down into bicarbonate; small amounts are also used in a variety of tissues by conversion back to pyruvate and processing in the Krebs cycle. Once produced in a cell, lactate can either be utilized by that cell or transported to the liver or other tissues for energy metabolism. Lactate will readily diffuse across cell membranes and into the cardiovascular circulation and has a very short half-life under normal conditions. Therefore, it serves as a rapid, inexpensive and reliable measure of tissue perfusion and cellular metabolism. In the critical patient, significant research has been performed in both the human and veterinary field demonstrating its utility as a marker of perfusion, as an indicator of sepsis and as a marker for response to goal-directed therapies. Blood lactate can also be used as a prognostic tool for survival and for the likelihood of development of complications and sepsis.

In the equine veterinary literature lactate has been extensively studied as a marker for prognosis, an indicator of tissue ischemia and sepsis, and a monitoring tool for response to therapy. Repeatable evidence has shown that comparison of lactate values from the jugular vein to other body cavities can have significant prognostic and diagnostic value. It has been shown in other veterinary species that lactate values may differ depending on the site of blood collection suggesting a significant effect of local production of lactate. However, limited research has been performed in the horse, which represents a gap in our knowledge. For example, one of the most active metabolic tissues in the horse is the lamina of the digit, therefore it is reasonable to suspect it could be a potential source of lactate production. Additionally, it is well established certain drugs, including α-2 agonists, can have profound suppressive effects on the cardiovascular system, which could decrease perfusion and increase lactate production.

These compounds are commonly used as part of standing sedation protocols in horses undergoing surgical procedures for prolonged periods. Available literature has only investigated the effects of short term (up to one hour) continuous use of these drugs on tissue metabolism. However, it is common for these procedures to far exceed one hour and there is limited information suggesting the effects. Therefore, as part of this degree, the purpose of the projects undertaken was to expand the current veterinary knowledge into these two regions of limited understanding utilizing lactate as the indicator of tissue metabolism and energy utilization.

The first study of this thesis compared jugular to cephalic venous lactate measurements in both healthy horses as well as in horses that presented with

significant clinical disease that commonly manifests with clinical hypoperfusion with and without sepsis. This study has shown that there does appear to be a significantly higher cephalic vein lactate concentration in clinically healthy horses compared to their jugular values. In all horses, both healthy and clinical cases, the cephalic lactate value was the higher value of the two sites. Additionally, horses that had poor disease outcomes (i.e. non-survivors and those that developed laminitis) failed to demonstrate a significant difference in their lactate values when comparing the jugular to the cephalic samples. This suggests that horses with severe clinical disease with poor outcomes will have a higher jugular lactate value than they would otherwise, making the difference between the jugular and the cephalic vein smaller. However, no difference was found between any sub-group of clinical cases and the trend in how their lactate values responded to treatment through time (within the first 24 hours). However, this study had several shortcomings, including a lack of consistency in timing of repeated sampling, small sample size in the clinical cases with poor disease outcomes and too short of a duration of sample collection. In addition, it was unexpected to find a significant difference between the jugular and cephalic vein lactate samples in clinically healthy horses. Combined these leave significant questions regarding the results and further study is warranted.

The second study built on findings from the first to study how perfusion parameters change over time in healthy horses that undergo prolonged sedation with the α-2 agonist, detomidine. Detomidine is a commonly used sedative and analgesic used in standing surgical procedures with known side effects on glucose metabolism, cardiovascular output, blood pressure and tissue perfusion. Standing surgical

procedures can require prolonged sedation (up to and exceeding four hours) so the purpose of the second project was to investigate how prolonged sedation can affect perfusion parameters and lactate production. This study demonstrated a statistically significant, but not clinically relevant elevation in lactate concentration as well as venous oxygen and carbon dioxide partial pressures. This demonstrates that detomidine, at the doses used in this study, does have a minor effect on tissue perfusion and therefore cellular metabolism. The only examined variable that had a clinically relevant change was glucose, where we report significant hyperglycemia. The blood glucose concentration consistently exceeded the renal threshold for glucose, therefore sustained hyperglycemia could potentially exacerbate hypoperfusion through osmotic diuresis. Of important consideration for this study was that the subjects did not appear to reach a plane of sedation that mimicked clinical settings, therefore additional studies are warranted to see if these parameters might change with more aggressive sedation protocols.

4.2 Future Directions

Despite earnest efforts to expanding the current knowledge in these respective areas of veterinary medicine several questions remain unanswered. The following areas outlined below serve as areas of additional focus for the author for continued research. These are points where the current study protocol was lacking or where the knowledge gained can be expanded.

Lactate comparison in normal healthy animals need more baseline of comparison to determine normal vs. abnormal. Due to the paucity of available data on this specific matter and given the information provided from our initial control sample of 10 healthy horses, additional sampling of healthy horses should be considered to determine a normal reference range for cephalic samples. This could provide useful information regarding a normal range, but also would provide data on the normal ratio of normal for comparison of jugular lactate values to cephalic. This could serve as a useful comparison in horses with clinical illnesses in a similar fashion as what has been previously identified when comparing the abdominal/thoracic cavity fluid to the jugular venous blood samples.

The need for horses with significant clinical disease to develop life threatening complications is an unfortunate desire when studying the prognostic potential of comparative samples to identify at risk horses. In the project detailed in Chapter 2, relatively few horses developed laminitis or other life threatening complications. This means that any statistical analysis related to the development of complications is also limited. Valuable information can be gained using models for complications (such as laminitis modeling) as a means of monitoring horses through a transition from healthy to diseased. Monitoring naturally occurring cases of laminitis and other complications would be better suited from an ethical standpoint however, this is reliant on treatment failure in a clinical caseload.

If significant information is gained from colitis cases, looking to see if this information is applicable across other types of systemic diseases (colic, peritonitis, pneumonia/pleuropneumonia, metabolic disease, grain overload, etc) would also be of

clinical interest. Expanding the inclusion criteria of clinical cases involved in additional studies may prove to be of benefit, especially to include diseases that involve sepsis or endotoxemia as part of the disease manifestation.

For the second study outlined in Chapter 3, additional investigation in the changes in lactate and glucose in horses administered a more clinically relevant dose of sedation that resembles the plane of sedation used during standing surgical procedures needs to be investigated. As some significant changes were noted during periods of maximal sedation, however beyond the first hour these horses did not appear to be significantly affected by their continuous infusions. In addition, there may be greater benefit in performing a study that samples hospitalized horses that are undergoing standing surgical procedures. This will also allow for investigation of additional variables that might have an effect of tissue perfusion such as increased sweat loss from impermeable drapes, additional medications such as opioids that can alter perfusion pressures, and abdominal insufflation. More sophisticated measuring tools of pressures, such as more accurate non-invasive blood pressure monitoring or the use of an arterial catheter, and use of a bench top blood gas analyzer validated for use in horses may also improve the quality of information obtained.

The two studies undertaken as part of this master's degree have demonstrated the clinical utility of lactate in equine veterinary medicine. Additional information regarding the lactate kinetics have been demonstrated in clinically diseased animals and in horses undergoing prolonged heavy sedation. However, there are still gaps in the available knowledge and while the projects undertaken here have demonstrated some interesting results, additional information is required to provide significant

guidelines that may help with clinical management of these cases in the future. Significant information can be gathered with minimal impact on these delicate patients with future studies and additional studies are warranted.