Establishing Prolonged Hyperlactatemia in a Canine Model

by

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Abstract

The purpose of this study was to maintain acid-base and electrolyte balance while inducing an eight-hour hyperlactatemic clamp of 8-9 mM using the canine model. Six adult dogs were anesthetized using sodium pentobarbital. Indwelling venous catheters were used for the infusion of 650 mM NaLa/HLa (pH 4.6), 80 mM KCl, and 42.5 mM $CaCl_2$. Arterial blood was collected using indwelling catheters for the analysis of PO_2, PCO_2, pH, $[K^+]$, $[Na^+]$, $[Ca^{2+}]$, [glucose], and [lactate]. All animals were mechanically ventilated throughout each experiment. Ventilation was decreased to allow hypercapnia for maintenance of normal arterial pH; and oxygen was supplemented for maintenance of normal arterial PO_2 levels. Infusion rates fo NaLa/HLa, KCl, and $CaCl_2$ were varied to maintain hyperlactatemia along with normal K^+, and Ca^{2+} levels. The pH of arterial blood fluctuated between 7.44 ± 0.02 and 7.48 ± 0.01. Arterial blood PCO_2 increased from 29 ± 5 mmHg at rest to 59 ± 5 mmHg by the end of infusion. Bicarbonate arterial concentrations increased from 20.9 ± 1.5 mM under control conditions to 39.1 ± 3.5 mM after NaLa/HLa infusion. Estimated base excess increased steadily from -2.22 ± 1.40 mM under control conditions to 14.23 ± 3.28 mM after NaLa/HLa infusion. Arterial PO_2 fluctuated between 96 ± 11 and 129 ± 19 mmHg throughout the ten-hour experiment. The arterial concentration of glucose fluctuated was maintained around 6.0 ± 0.6 mM throughout the experiment. Sodium concentration in arterial blood rose from 142 ± 1 mM under control conditions to a max concentration of 154 ± 7 mM by nine hours of

infusion. Arterial K^+ and Ca^{2+} concentrations decreased slightly throughout the experiment from 3.3 ± 0.2 to 2.9 ± 0.4 mM and 1.28 ± 0.09 to 1.16 ± 0.19 mM, respectively. Blood lactate concentration was successfully increased from 0.7 mM to approximately 8.0-9.0 mM over an eight-hour period. Infusion of K^+ and Ca^{2+} with permissive hypercapnia were required to maintain electrolyte and pH values within the physiological range. This is the first study to maintain hyperlactatemia for greater than three hours without the disturbance of acid-base or ion balance.

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ADP	adenosine diphosphate
AMP	adenosine monophosphate
ANLSH	astrocyte-neuron lactate shuttle hypothesis
ARDS	acute respiratory distress syndrome
ATP	adenosine triphosphate
BE	base excess
CA	carbonic annhydrase
Ca^{2+}	ionized calcium
$CaCl_2$	calcium chloride
CNS	central nervous system
CO_2	carbon dioxide
CSF	cerebral spinal fluid
$ECCO_2R$	extracorporeal carbon dioxide removal
ECF	extracellular fluid
FDGPET	fluorodeoxyglucose positron emission tomography
FFAs	free fatty acids
FPI	fluid percussion injury
FT	fast twitch
H^+	hydrogen ion

HCO_3^-	bicarbonateHLa lactic acid
H-type LDH	heart-type lactate dehydrogenase
IMP	inosine monophosphateiPO_2 intracellular partial pressure of oxygen
K^+	potassium
KCl	potassium chloride
kg	kilogram
L	liter
$L \cdot min^{-1}$	Liter per minute
La^-	lactate
LDH	lactate dehydrogenase
LPH	lactate protected hypoglycemia
MCT	monocarboxylate transport protein
mEq	milliequivalents
mmHg	millimeters of mercury
min	minute
ML	membrane lung
ml	milliliters
mmol	millimoles
mM	millimolar
μmol	micromoles
molar	M
m-type LDH	muscle-type lactate dehydrogenase
MWM	Morris water maze

Na^+	sodium
NADH	reduced form of nicotinamide adenine dinucleotide
NaLa	sodium lactate
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
N	normal = Equivalents/Liter
O_2	oxygen
PCO_2	partial pressure of carbon dioxide
PEPCK	phosphoenolpyruvate carboxykinase
PFK-1	phosphofructokinase-1
P_i	inorganic phosphate
pK_a	negative logarithm of the acid dissociation constant
PO_2	partial pressure of oxygen
RMANOVA	repeated measures analysis of variance
SID	strong ion difference
ST	slow twitch
TBI	traumatic brain injury
$\dot{V}CO_2$	volume of carbon dioxide produced per minute
$\dot{V}O_2$	volume of oxygen consumed per minute
$\dot{V}O_{2max}$	maximum O_2 consumption per minute
3MP	3-mercaptopicolinate

1. REVIEW OF LITERATURE

Lactic Acid versus Lactate

The negative logarithm of the dissociation constant (pK_a) of lactic acid is 3.87 at 37˚C (Nims & Smith, 1936; Martin & Tartar, 1937; Roos, 1975) and the pH of arterial blood and muscle at rest are 7.4 (Adeva-Andany *et al.*, 2014) and 7.01 (Pan *et al.*, 1988) respectively, with muscle pH reaching as low as 6.1 during exhaustive exercise (Pan *et al.*, 1988). Since the pH of blood and muscle is much higher than the pK_a value of lactic acid, greater than 99% of lactic acid in the blood and muscle is dissociated, forming a lactate anion (La^-) and proton (H^+) (Gladden, 2004a, b). Throughout this review the term lactate, instead of lactic acid, will be used since this is a more physiologically correct term.

Brief History of Lactate

In 1780 lactate was first isolated in sour milk by the Swedish chemist, C. W. Scheele (Kompanje *et al.*, 2007). Twenty eight years after Scheele's discovery of lactate, J.J. Berzelius was the first to discover lactate in the fluid of extracted meat (Kompanje *et al.*, 2007). Then, in 1843 J. J. Scherer discovered the presence of lactate in human blood after death (Kompanje *et al.*, 2007); however, it was not until 1858 that lactate was discovered in the blood of a living patient by C. Folwarczny (Mosler & Körner, 1862). Following Folwarczny's discovery, Du Bois-Reymond in 1859 noted that activity causes muscles to become acidic, likely induced by lactate (von Muralt, 1950). In 1864 Heidenhain was the first to report that the amount of lactate in muscle increased in

proportion to the amount of work being done by the muscle (von Muralt, 1950). This discovery led to the question as to why muscle work causes the formation of lactate.

Lactate Accumulation, Oxygen, and Muscle Contraction/Exercise

Since bacteria produce lactate anaerobically (Garvie, 1980) it was logical to assume the converse; that is that the presence of lactate indicates a lack of oxygen (O_2). Therefore, many assumed that lactate accumulation during exercise must be caused by a lack of O_2 in the exercising muscles. This notion of lactate being formed due to the absence of oxygen dates back to the work of Louis Pasteur in the nineteenth century (Brooks, 2009). In 1891 Araki and Zellessen found that if the oxygen supply to muscles in mammals and birds was interrupted, lactate was formed; further supporting the notion that a lack of muscle oxygen causes the formation of lactate (Kompanje *et al.*, 2007). To further support the idea that a lack of oxygen causes lactate accumulation, Fletcher and Hopkins discovered in 1907 that when fatigued amphibian muscles containing high concentrations of lactate were placed in oxygen rich environments, the lactate disappeared (Fletcher & Hopkins, 1907). A.V. Hill was also in agreement with hypoxia being the cause of lactate production, and in 1924 suggested that lactate accumulates in active muscle and then diffuses into blood due to a lack of muscle O_2 during exercise (Hill *et al.*, 1924).

A paradigm shift began with a study by Welch and Stainsby in 1966, in which they observed that the gastrocnemius-plantaris muscle group in humans produced small amounts of lactate at rest (Stainsby & Welch, 1966). Stainsby and Welch also discovered that supramaximal contractions of the gatrocnemius-plantaris muscle group induced by electrical stimulation of 0.5-10 impulses/second caused lactate production to increase

transiently and then decrease as contractions continued (Stainsby & Welch, 1966). The authors concluded that the transient nature of lactate production may be caused by aerobic glycolysis or that O_2 supply may be inadequate transiently and lactate formed as a result of dysoxia (inadequate oxygen for aerobic energy production) (Stainsby & Welch, 1966). Jöbsis and Stainsby (1968) were the first to argue against the idea that muscle dysoxia causes increased blood lactate concentrations during submaximal exercise (Gladden, 1996). They speculated that lactate formation would be accompanied by increased levels of the reduced form of nicotinamide adenine dinucleotide (NADH) if hypoxia was indeed the cause of lactate formation (Jöbsis & Stainsby, 1968). Upon stimulation of dog gastrocnemius and gracilis muscles at supramaximal rates, the signal from their surface fluorometric technique revealed oxidation of NADH to NAD^+ indicating that oxidative phosphorylation was occurring concurrent with lactate formation (Jöbsis & Stainsby, 1968). This finding supported the notion that lactate is not only produced in response to dysoxia, but also when O_2 is in ample supply for aerobic energy production.

In 1983, Connett and colleagues postulated that various signals other than dysoxia may stimulate glycolysis; and that although the accumulation of lactate may be caused by dysoxia, lactate may not necessarily indicate the occurrence of tissue dysoxia (Connett *et al.*, 1983). Connett et al. (1986) used a canine model involving contraction of the gracilis muscle from 10-100% maximum O_2 consumption per minute ($\dot{V}O_{2max}$) while calculating the intracellular partial pressure of O_2 (iPO_2) from the oxymyoglobin-dissociation curve (Connett *et al.*, 1986). They demonstrated that muscle lactate production was correlated with the volume of oxygen consumed per minute ($\dot{V}O_2$) but was not correlated with

intracellular partial pressure of O_2 (iPO_2) (Connett *et al.*, 1986). More recent studies have confirmed Connett's hypothesis indicating that lactate efflux with increasing work rate is not the result of inadequate cellular O_2 (Richardson *et al.*, 2006), and decreases in iPO_2 do not limit $\dot{V}O_2$ by exercising muscle (Molé *et al.*, 1999). Although it is unlikely that a low iPO_2 causes lactate formation by muscle it is possible that during moderate to intense exercise there could be dysoxic loci (iPO_2 of 1-2 millimeter of mercury (mmHg)) in the mitochondria that are missed when studying whole muscle responses (Clanton *et al.*, 2013).

The evidence above suggests that oxidative phosphorylation is not O_2-limited at metabolic rates associated with net lactate production and accumulation, and that mitochondria do not have to be dysoxic for muscle cells to produce lactate. It is now widely accepted that the primary mechanisms responsible for increasing muscle and blood lactate concentrations, are accelerated glycolysis, an increase in fast twitch (FT) muscle fiber recruitment, and a failure of lactate removal mechanisms to match the rate of lactate production (Gladden, 2004b; Clanton *et al.*, 2013). The lactate threshold during incremental exercise, defined as the exercise intensity at which blood lactate levels increase exponentially (Gladden, 1984) is attributed to these three factors.

Lactate production in the cell is dependent upon the fate of NADH. Once NADH is formed in glycolysis by the glyceraldehyde 3-phosphate dehydrogenase enzyme it can either be shuttled into the mitochondria of the cell where it can be used to form adenosine triphosphate (ATP) via oxidative phosphorylation or it can be oxidized in the cytosol by lactate dehydrogenase (LDH) producing lactate from pyruvate (Huckabee, 1958). Lactate is formed readily during glycolysis because LDH catalyzes a near-equilibrium

reaction and is located in the cytosol of the cell where it has easy access to its substrate, pyruvate (Gladden, 2004a).

During muscle contraction the rate of glycolysis is increased due to the activation of glycolytic regulatory enzymes. Phosphorylase is a regulatory enzyme that breaks down muscle glycogen to be used in glycogenolysis and is activated by calcium (Ca^{2+}), epinephrine, adenosine monophosphate (AMP), adenosine diphosphate (ADP), inorganic phosphate (P_i), and inosine monophosphate (Cohen *et al.*, 1967; De Campos, 2000). The other key enzyme in controlling the speed of glycolysis is phosphofructokinase-1 (PFK-1), which is activated by ADP, AMP, and P_i (Lowry & Passonneau, 1966; Layzer *et al.*, 1969; Tornheim & Lowenstein, 1976). Calcium, epinephrine, AMP, ADP, P_i, and IMP all increase during exercise in proportion to exercise intensity (Dobson *et al.*, 1971; Ljunghall *et al.*, 1984; Kjaer, 1989; Sahlin & Broberg, 1990). Since the concentrations of glycolytic activators rise as exercise intensity increases, so also does the rate of glycolysis, causing an increase in lactate production.

Another cause for an increased rate of glycolysis as exercise intensity increases has to do with the type of motor units recruited during exercise. At low intensity exercise slow twitch (ST) motor units are recruited, but as exercise intensity increases there is a progressive recruitment of fast twitch (FT) motor units (Henneman *et al.*, 1965; Burke & Edgerton, 1975; Burke, 2011). Research has shown that a greater maximal rate of lactate is produced from FT than ST muscle fibers (Spriet *et al.*, 1987). The increased rate of lactate production by FT muscle fibers has been correlated with a greater total activity of muscle (M)-type LDH, than the heart (H)-type LDH; whereas ST muscle fibers have been found to have a greater total activity of the H-type, than M-type LDH (Kaplan &

Everse, 1972; Van Hall, 2000). However, attributing the increased lactate production in FT muscle fibers to the prevalence of M-type LDH may be misguided, since LDH catalyzes a near-equilibrium reaction (Gladden, 2004a).

In continued support of lactate being more readily produced by FT muscle fibers, the glycolytic enzymes phosphorylase, PFK, and LDH have been shown to have higher activity in FT than in ST muscle fibers (Tesch, 1980). Another reason glycolytic rate may be higher in FT than ST muscle fibers is due to the lower oxidative capacity of FT fibers marked by a lower mitochondrial density (Kirkwood *et al.*, 1986), lower capillarity (Andersen, 1975), and lower myoglobin concentration (Peter *et al.*, 1972) compared to ST muscle. A lower mitochondrial density, capillarity and myoglobin concentration limits the rate of mitochondrial respiration causing a buildup of NADH in the cytosol of FT muscle cells increasing the need of lactate production and oxidation of NADH to NAD^+ for the acceptance of electrons at the glyceraldehyde 3-phosphate dehydrogenase step of glycolysis so that glycolysis can continue. Whether or not it is the predominant type of LDH or the metabolic profile, FT fibers have been shown to be activated during high intensity exercise and to produce a greater amount of lactate than ST fibers.

A third cause for increased lactate levels during exercise is that increases in epinephrine concentration cause a decrease in lactate uptake by contracting muscles (Hamann *et al.*, 2001). Furthermore, the sympathetic nervous system plays a major role in redistributing blood flow away from inactive tissues to active skeletal muscle (Nielsen *et al.*, 2002). As a result, blood flow to potential lactate removal sites such as the liver, kidneys, and inactive muscle may be reduced as exercise intensity increases (Rowell,

1974). A decrease in lactate uptake can result in less gluconeogenesis from lactate, less lactate oxidation, and less total lactate removal, leading to increased blood lactate levels.

Throughout the years of studying lactate production by skeletal muscle it is now widely accepted that hypoxia is unlikely to be the *primary* cause of lactate accumulation during mild to moderate exercise (at and below the lactate threshold) in normal ambient conditions. Production of lactate oxidizes NADH to NAD^+ so that glycolysis can continue; however, lactate can also play a role as a fuel source and signaling molecule during and after exercise.

Lactate as a Fuel Source and Signaling Molecule

Historically, skeletal muscle has been considered a site of lactate production due to muscle contraction and a lack of O_2. However, it was later recognized that skeletal muscle contains the necessary mechanisms for lactate removal (Fletcher & Hopkins, 1907). Since this discovery, it has been observed that both contracting skeletal muscle (Newman *et al.*, 1937; Stainsby & Welch, 1966; Freyschuss & Strandell, 1967; Hermansen & Vaage, 1977; Dodd *et al.*, 1984; Katz, 1986; Richter *et al.*, 1988; Gladden, 1991; Bergman *et al.*, 1999; Van Hall *et al.*, 2003; Menzies *et al.*, 2010) and resting muscle (Freyschuss & Strandell, 1967; Ahlborg *et al.*, 1975) can take up and oxidize lactate. Direct calorimetry has revealed that about 4.84 kcals are available per liter of O_2 consumed for lactate, and about 4.72 kcals for fats, indicating about a 7% advantage in moles ATP/mole O_2 for using lactate as a fuel in comparison to free fatty acids (Stanley *et al.*, 2005; Clanton *et al.*, 2013). Brooks has also reported evidence that lactate flux and oxidation can equal or exceed glucose flux and oxidation during exercise (Brooks, 2000).

Lactate can also be used by the liver, kidneys, and heart. Both the liver and kidneys can use lactate to produce glucose in a process known as gluconeogenesis (Cori, 1931; Benoy & Elliott, 1937; Drury *et al.*, 1950; Krebs *et al.*, 1966; Waterhouse & Keilson, 1969). The heart, on the other hand, can use lactate as a fuel source and has been shown to take up lactate in direct proportion to plasma lactate concentration (Drake *et al.*, 1980). Lactate uptake by the heart has also been shown to increase with exercise as the concentration of lactate in the blood rises (Gertz *et al.*, 1988; Kemppainen *et al.*, 2002).

In addition to skeletal and cardiac muscle, neurons have also been shown to take up and oxidize lactate as a fuel source. The traditional view has been that neuronal energy metabolism is fueled solely by glucose oxidation (Chih *et al.*, 2001). This conventional perspective was challenged by Pellerin and Magistretti with their introduction of the astrocyte-neuron lactate shuttle hypothesis (ANLSH), which states that astrocytes produce lactate and neurons take up that lactate as a fuel source (Pellerin & Magistretti, 1994).

Since this model was proposed, further research has shown that neuronal tissue can use lactate as a fuel source in animals. In the absence of glucose, lactate has been found to sustain function in myelinated axons of the central nervous system (CNS) in rats (Brown *et al.*, 2001). Additional studies on rats, using ^{13}C- nuclear magnetic resonance (NMR) *in vitro* showed that neurons prefer lactate to glucose as a fuel source when glucose and lactate concentrations are identical (Bouzier-Sore *et al.*, 2003) and the vagus nerve of rabbit has been found to oxidize lactate more rapidly than glucose (Véga *et al.*, 1998). In mice there is evidence that lactate is produced by astrocytes and that cortical

neurons take up lactate as a fuel; i.e., lactate is interchangeable with glucose as a fuel (Pellerin *et al.*, 1998). This is in agreement with other studies that have shown lactate to sustain rates of oxidation superior to glucose in synaptosomes (McKenna *et al.*, 1994) and that lactate alone is capable of fully supporting synaptic transmission (Schurr *et al.*, 1988).

In addition to studies on animals, there have also been studies showing that the human brain can oxidize lactate as a fuel source *in vivo*. Intravenous lactate infusion raising blood lactate levels to 3.3 millimolar (mM) has been shown to prevent cerebral dysfunction during hypoglycemia as low as 2.5 mM glucose (Maran *et al.*, 1994; Veneman *et al.*, 1994; King *et al.*, 1998). Also, global brain studies *in vivo* on euglycemic humans using ^{18}fluorodeoxyglucose positron emission tomography (FDGPET) show that when lactate levels are elevated, glucose uptake is significantly reduced (Smith *et al.*, 2003). Along with lactate being a substrate for most body tissues, it is also a signaling molecule sometimes referred to as 'lactormone' (Brooks, 2009).

Lactate formation is the result of reducing pyruvate so that when lactate is oxidized back to pyruvate cell redox balance is changed (Brooks, 2009). Therefore, when lactate is released by one cell and taken up by another through monocarboxylate transport proteins (MCTs) via the cell-cell shuttle (Brooks, 1998), the oxidation of lactate represents a major signaling mechanism caused by the redox changes (Brooks, 2009). When blood lactate concentrations are increased, redox changes may cause lactate to be substituted as a major energy substrate and even down-regulate the use of other energy substrates (Brooks, 2009). Indeed, the increase of blood lactate levels has been shown to increase lactate disposal and oxidation, while decreasing the disposal and oxidation of

glucose and free fatty acids (FFAs) (Gertz *et al.*, 1988; Saddik *et al.*, 1993; Brooks, 1998; Miller *et al.*, 2002a; Miller *et al.*, 2002b; Liu *et al.*, 2009).

Lactate may also produce long-term changes by affecting gene expression. It has long been known that endurance training stimulates mitochondrial biogenesis (Holloszy, 1967), and increased monocarboxylate transporter 1 (MCT1) expression has also been shown to correlate with levels of mitochondrial proteins (Brooks, 1985). Recent research has shown that *in vitro* lactate appears to be a signaling molecule that can affect its own metabolism by increasing transcription factors for MCT1 expression and mitochondrial biogenesis (Hashimoto *et al.*, 2007; Brooks, 2009).

Since its discovery in 1780, lactate has been researched extensively. It is now known that lactate is typically found in blood and muscle dissociated from its proton as lactate and H^+, the majority of cells in the body can use lactate as a fuel source, and lactate can also act as a signaling molecule. Recently, lactate has received significant attention from a medical perspective and may be used as a treatment for patients with cancer, acute respiratory distress syndrome (ARDS), and traumatic brain injury (TBI).

Lactate and the Warburg Effect

Most normal cells (although there are exceptions) primarily rely on mitochondrial oxidative phosphorylation to generate the energy needed to carry out cellular processes. However, many cancer cell types rely on aerobic glycolysis, termed the Warburg effect (Warburg, 1956) to generate energy. This means that even in conditions with ample O_2 available, cancer cells undergo rapid glycolysis, producing and releasing large amounts of lactate.

Since cancer cells rely heavily on glucose to proliferate and maintain viability, starving cancer cells of glucose may kill tumor cells or make current treatment such as chemotherapy more effective. Using a murine (pro-B-cell) FL5.12 cell line, it has been found that withdrawing glucose induces cell death (Vander Heiden *et al.*, 2001). Although decreasing glucose availability *in vitro* is effective in decreasing the viability of cancer cells, hypoglycemia *in vivo* can be lethal to the organism as a whole. This understanding has led to the search for a salvage fuel that non-malignant cells can use, while glucose is depleted to treat cancer cells (Nijsten & van Dam, 2009).

Burt et al. used glycerol as a salvage fuel during hypoglycemia in rats and one cancer patient (Burt *et al.*, 1985). Hypoglycemia was achieved by administration of 3-mercaptopicolinate (3MP) which is an inhibitor of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) (Burt *et al.*, 1985). However, infused glycerol enters the gluconeogenic pathway distal to PEPCK so this treatment had no effect on decreasing blood glucose levels (McCarty, 2001). Lactate is another potential salvage fuel since many normal cells can metabolize lactate under aerobic conditions whereas cancer cells exhibiting the Warburg effect are net lactate producers, not consumers, even under aerobic conditions. Hypoglycemic counter regulation (gluconeogenesis) has also been shown to be attenuated with lactate infusion in rats (Borg *et al.*, 2003), suggesting that lactate may be a useful salvage fuel without disrupting the hypoglycemic condition.

In humans, a lactate concentration of 2.4 mM has been shown to have a protective effect on the brain during hypoglycemia (5-2.4 mM glucose) for at least 3.5 hours (Maran *et al.*, 1994). Nijsten et al. have hypothesized that local or systemic induction of

hypoglycemia with concurrent lactate infusion will adversely affect tumors displaying the Warburg effect while leaving normal cells unharmed (Nijsten & van Dam, 2009). In addition to a potential treatment for cancer, lactate infusion has also been hypothesized as a treatment for reducing PCO_2 in patients with ARDS thereby decreasing mechanical ventilatory rate and reducing lung injury.

Lactate and Extracorporeal Carbon Dioxide Removal

Patients with ARDS have an increased ventilatory dead space, which impairs the clearance of carbon dioxide (CO_2) from the blood (Nuckton *et al.*, 2002). In order to maintain physiological blood pH and partial pressure of carbon dioxide (PCO_2), patients with ARDS must be ventilated mechanically to increase minute ventilation (De Campos, 2000); however, mechanical ventilation has been shown to cause lung injury (Mascheroni *et al.*, 1988).

Using a membrane lung (ML) outside of the body has been proposed as a mechanism to assist in the removal CO_2, thereby allowing a lower ventilatory rate and reducing the risk of ventilator-induced lung injury (Terragni *et al.*, 2009; Bein *et al.*, 2013). This mechanism has been termed extracorporeal carbon dioxide removal ($ECCO_2R$). Currently, extracorporeal blood flow must be in the range of 0.5 to 1 Liter per minute ($L \cdot min^{-1}$), requiring the use of large gauge catheters (Pesenti *et al.*, 2010; Crotti *et al.*, 2012) which limits the use of $ECCO_2R$ to major medical centers and severely ill patients (Zanella *et al.*, 2014). Due to this limitation it is necessary to develop a technique that would allow the use of $ECCO_2R$ at blood flows of 0.2-0.4 $L \cdot min^{-1}$ expanding the relevance of $ECCO_2R$ beyond solely preventing ventilatory-induced lung injury in patients with severe ARDS (Patroniti *et al.*, 2011). Reducing rates

of endotracheal intubation may decrease the incidence of ventilator-associated pneumonia, sedation requirements, and patient discomfort (Zanella *et al.*, 2014).

It has been hypothesized that adding acid into the blood entering the ML would increase CO_2 removal by converting bicarbonate (HCO_3^-) ions into CO_2 thereby increasing the PCO_2 transmembrane gradient and subsequent CO_2 transfer (reaction 1) (Zanella *et al.*, 2009). The use of lactic acid infusion of 1, 2, and 5 milliequivalents (mEq)/min in the blood entering the ML has been shown to increase CO_2 removal by 11, 23, and 70% respectively in swine; supporting the hypothesis that increasing the concentration of acid entering the ML improves the removal of CO_2 by the ML (Zanella *et al.*, 2009). However, lactic acid infusion was only sustained for 15 minute intervals with 30 minutes of rest between experiments making it unclear if lactic acid infusion can be used for extensive periods of time (Zanella *et al.*, 2009).

Carbonic Anhydrase (CA) reaction:

$$H^+ + HCO_3^- \xrightarrow{CA} H_2CO_3 \leftrightarrow CO_2 + H_2O \quad \text{.....reaction 1}$$

In a follow up study, Zanella et al. extended the period of lactic acid infusion to 48 hours (Zanella *et al.*, 2014). This study showed that over 48 hours arterial pH decreased slightly by about 0.04 pH units while arterial lactate levels remained below 4 mEq/Liter (L) (Zanella *et al.*, 2014). Infusion of lactic acid at the ML increased PCO_2 providing safe removal of CO_2 using only 250 milliliters (ml)$\cdot min^{-1}$ extracorporeal blood flow and no signs of organ or red blood cell damage were discovered throughout the protocol (Zanella *et al.*, 2014). These studies have shown that adding acid to extracorporeal blood can improve CO_2 removal by a membrane lung. However, the metabolic impact of lactic acid infusion must be studied further (Zanella *et al.*, 2014).

Lactate and Traumatic Brain Injury

The role of lactate infusion in the treatment of cancer and prevention of lung injury from mechanical ventilation in patients with ARDS has been discussed. In addition to these situations, patients who have experienced brain trauma may also benefit from lactate infusion. This section of the review will discuss the possible use of lactate infusion as a treatment for TBI.

Traumatic brain injury causes over 50,000 deaths and permanently disables another 70,000 to 90,000 individuals every year (Thurman & Guerrero, 1999). Although patients with moderate head injuries may appear to recover fully, the injury typically results in long lasting negative effects such as forgetfulness, slower processing of ideas, irritability, and the inability to concentrate (Hilton, 1994). Traumatic brain injury is a multifaceted process which includes ischemia, high intracellular calcium levels, and impaired mitochondrial function (Rice *et al.*, 2002).

As discussed earlier in this review, it is known that the brain can use lactate as a fuel source in replacement of glucose (Schurr *et al.*, 1988; McKenna *et al.*, 1994; Pellerin & Magistretti, 1994; Pellerin *et al.*, 1998; Véga *et al.*, 1998; Brown *et al.*, 2001; Chih *et al.*, 2001; Bouzier-Sore *et al.*, 2003). Recently there has been additional evidence that the brain may preferentially metabolize lactate in response to TBI indicated by increased cerebral spinal fluid (CSF) and extracellular fluid (ECF) lactate concentrations in the injured brain following trauma (King *et al.*, 1974; Inao *et al.*, 1988). This increased level of lactate in the brain may be beneficial as studies have reported that lactate primarily fuels recovery of synaptic function upon reoxygenation after hypoxia in the rat (Schurr *et al.*, 1988; Schurr *et al.*, 1997) and that increasing brain lactate levels results in better

recovery following fluid percussion injury (FPI) in rats than if brain glucose levels are increased (Chen *et al.*, 2000a). Chen et al. used ^{14}C-lactate to test whether or not lactate is acutely taken up by the injured brain after FPI in the rat (Chen *et al.*, 2000b). The results of this study showed that the uptake of ^{14}C-lactate was 2.5 times greater at the injury site compared to uninjured brain tissue, suggesting that the injured brain may use lactate as an energy source (Chen *et al.*, 2000b). This study has led to the hypothesis that lactate administration early after TBI may benefit the injured brain by improving Krebs Cycle metabolism and restoring ionic homeostasis (Chen *et al.*, 2000b).

Rice et al. conducted a study to test their hypothesis that astrocytes take up glucose and produce lactate for use by neurons, and by providing exogenous lactate the damaged neurons will recover quicker or not have as many disruptions of synaptic connections (Rice *et al.*, 2002). To test this hypothesis a lateral FPI to the hippocampus was induced in anesthetized rats. Thirty minutes following lateral FPI, 100 mM L-lactate or saline was administered via the jugular vein for 3 hours at an infusion rate of 0.65 ml/hr. Days 11-15 following treatment the rats were tested using the Morris water maze (MWM) for assessment of cognitive deficits, since the hippocampus is associated with memory and learning. The results showed that injured rats treated with lactate performed significantly better compared to the injured rats treated with saline. Rice and colleagues concluded from the results of this study that lactate infusion has potential benefits in the treatment of moderately brain injured patients, when administered in an emergency room setting. However, since severely head injured patients typically present to the clinic with lactic acidosis lactate infusion may not be recommended in the scenario of severe head trauma (Rice *et al.*, 2002). In any case, further study on the effects of acid-base balance

from lactate infusion must be further studied before this type of treatment can be put into practice.

Methodological Aspects of Lactate/Lactic Acid Infusion

Lactic acid has two opposing effects on blood pH. It has an acidifying effect due to its dissociation properties as a weak acid (Schwartz & Waters III, 1962; Gladden & Yates, 1983) and its increase of the strong ion difference (SID) (Stewart, 1983). An alkalinizing effect is caused by the oxidation of lactic acid to CO_2 and $H_2O + HCO_3^-$ or formation of glycogen + HCO_3^- and a concomitant decrease in the SID (Schwartz & Waters III, 1962; Gladden & Yates, 1983; Stewart, 1983). Therefore any neutralization of a lactic acid infusate will diminish its acidifying effect and increase its alkalinizing effect (Gladden & Yates, 1983). For many years, sodium lactate (NaLa) and lactic acid (HLa) have been used for infusion; however, maintaining acid balance during infusion is quite difficult. The following studies have used NaLa infusion to study the effect of NaLa on acid-base balance and to study lactate metabolism.

Cohen et al. performed a study in which six dogs were infused with NaLa to study its effect on intracellular pH (Cohen *et al.*, 1967). An infusion of 4.8 millimoles (mmol)·kilogram $(kg)^{-1}$ of isotonic (163 mM) NaLa (pH 7.0) was given for 17 minutes (Cohen *et al.*, 1967). PCO_2 was maintained constant throughout the experiment around 40 mmHg via mechanical ventilation (Cohen *et al.*, 1967). Arterial blood lactate concentration increased from 0.55 mM to 3.5 mM during NaLa infusion, while arterial blood pH increased from 7.32 before NaLa infusion to 7.50 after infusion (Cohen *et al.*, 1967).

Karetzky and Cain infused anesthetized dogs with NaLa to study oxygen uptake in response to NaLa infusion (Karetzky & Cain, 1969). The NaLa infusion protocol consisted of infusing 10 dogs with a concentration of 0.3 molar (M) NaLa (pH 7.0) at 3 $mmoles \cdot kg^{-1}$ for 10 minutes with a 60 minute recovery period (Karetzky & Cain, 1969). Arterial lactate concentration rose rapidly with NaLa infusion from 1 mM to 10 mM (Karetzky & Cain, 1969). After NaLa infusion arterial PCO_2 rose slightly (~ 42 mmHg) and rapidly returned toward its control value of 40 mmHg (Karetzky & Cain, 1969). Arterial HCO_3^- concentration increased from 20 mM to about 24 mM 30 minutes post NaLa infusion, while the arterial pH concentration rose from 7.32 to 7.38 (Karetzky & Cain, 1969).

Ahlborg et al. infused ten healthy male volunteers with a 20 to 40 mM (pH 7.0) NaLa bolus and then infused 4 $mmoles \cdot ml^{-1}$ of NaLa at rates of 5 to 12 $mmol \cdot kg^{-1}$ intravenously for 30 minutes to determine the influence of lactate on fuel metabolism and blood flow (Ahlborg *et al.*, 1976). Arterial lactate levels increased from 0.55 mM to 5 mM during the infusion period (Ahlborg *et al.*, 1976). The authors acknowledged that an increase in pH likely took place during the study due to NaLa infusion, but did not make any pH measurements (Ahlborg *et al.*, 1976).

Ryan et al. (1979) infused NaLa at a rate of 0.05 $mmol \cdot kg^{-1} \cdot min^{-1}$ (pH 7.0) for 20 minutes during rest and then 20 minutes during submaximal exercise in six male volunteers to study the body's ability to metabolize lactate during rest and exercise (Ryan *et al.*, 1979). Plasma lactate levels in the subjects increased from a 1.3 mM baseline to 4.8 mM during NaLa infusion at rest and 9.1 mM by the end of exercise (Ryan *et al.*, 1979). Plasma HCO_3^- levels increased in all subjects by an average of 1.8 mM under

resting infusion and by 6.1 mM with infusion during exercise. The volume of carbon dioxide produced per minute ($\dot{V}CO_2$) increased by 0.026 L/min during resting infusion, but decreased by 0.060 L/min with exercise, and arterial PCO_2 remained unchanged during resting and exercise NaLa infusion (Ryan *et al.*, 1979). Arterial blood pH was measured, but not reported in this study. However, pH likely increased due to the metabolism of lactate decreasing, the SID and producing HCO_3^- ions.

Chioléro et al. infused 0.5 M NaLa (pH 7.0) at a rate of 20 micromoles (μmol)·kg^{-1}·min^{-1} into an indwelling catheter in an antecubital vein for 3 hrs in six healthy human volunteers under supine rest (Chioléro *et al.*, 1993). Baseline plasma lactate levels were 1.9 mM and were raised to 3.7 mM after 3 hrs (Chioléro *et al.*, 1993). The $\dot{V}CO_2$ decreased from 0.203 L/ minute to 0.192 L/min with lactate infusion indicating that arterial PCO_2, although not measured, likely increased during the 3 hr experiment (Chioléro *et al.*, 1993). Venous plasma bicarbonate increased from 24.9 mM to 32.3 mM after 3 hrs of NaLa infusion and venous plasma pH increased from 7.38 to 7.47 after the 3 hrs of lactate infusion (Chioléro *et al.*, 1993).

Ferrannini et al. infused a 2 M concentration of NaLa (pH 6.7) at 25 μmol·kg^{-1}·min^{-1} into an antecubital vein for a total of three hours in 12 human subjects resting supine (Ferrannini *et al.*, 1993). Blood lactate concentrations rose from 0.8 mM to 2.9 mM by the end of three hours (Ferrannini *et al.*, 1993). The $\dot{V}CO_2$ did not change during the experiment (Ferrannini *et al.*, 1993). Arterial blood HCO_3^- increased from 24.5 mM to 31.2 mM, while arterial pH increased from7.37 to 7.45 by the end of three hours NaLa infusion (Ferrannini *et al.*, 1993).

As these studies have shown, infusing NaLa causes an alkalotic effect, increasing blood pH due to the metabolism of lactate. Although there have been many studies employing NaLa infusion in humans and animals there are few studies that have employed the infusion of HLa. The following two studies demonstrate the role that lactic acid infusion plays on blood acid-base balance.

Yudkin and Cohen infused HLa into the jugular vein of rats (Yudkin & Cohen, 1975). A load of 8.9 $mmol \cdot kg^{-1}$ of L(+)-lactic acid (3.6 M, pH 7.0) was infused with a constant-infusion pump though the cannula in the left jugular vein over a 10 min period (Yudkin & Cohen, 1975). Blood lactate concentration increased from 1 mM to 16 mM and pH decreased from 7.48 to 7.26 during infusion (Yudkin & Cohen, 1975).

Gonzalez et al. infused nephrectomized dogs with 25-30 $ml \cdot kg^{-1}$ of 0.3 M HLa initially, followed by a lower infusion to maintain a constant arterial pH for 30-40 min (Gonzalez *et al.*, 1976). Arterial PCO_2 remained constant (35-40 mmHg) throughout the study (Gonzalez *et al.*, 1976). However, arterial blood HCO_3^- concentration decreased from 22 mM to 10 mM and arterial pH also decreased from 7.40 to 7.10 (Gonzalez *et al.*, 1976).

These studies using NaLa and HLa infusion confirm that lactic acid can cause alkalosis or acidosis depending on infusate pH. Due to the complexity of maintaining blood acid-base balance while infusing NaLa and HLa, Gladden and Yates conducted a study in 14 anesthetized dogs to determine the optimal infusate pH to maintain normal blood acid-base balance (Gladden & Yates, 1983).

In the Gladden and Yates study, sodium lactate/lactic acid with pH varying from 2.0-7.0 at a concentration of 350-400 mM was infused at a rate of 2.0 $ml \cdot kg^{-1} \cdot min^{-1}$ (0.75

mmol·kg^{-1}·min^{-1}) for the 1st minute, 1.0 ml·kg^{-1}·min^{-1} (0.38 mmol·kg^{-1}·min^{-1}) for the next 9 min, and 0.2 ml·kg^{-1}·min^{-1} (0.08 mmol·kg^{-1}·min^{-1}) for the remaining 50 min of the study into the right brachial vein of dogs (Gladden & Yates, 1983). No respiratory compensation to manipulate arterial blood pH was performed during infusion (Gladden & Yates, 1983). Arterial blood lactate concentration was increased from 1.4 mM during control conditions to 11.0 mM after 20 min of NaLa/HLa infusion; however, arterial blood lactate concentration decreased significantly to 9.8 mM after 30 min of infusion and remained approximately 9.8 mM for the rest of the experiment (Gladden & Yates, 1983).

Above an infusate pH of 4.4, arterial pH increased by 0.118-0.167 during infusion; the arterial pH was unchanged (0.00-0.056) when the infusate pH was between 3.4 and 4.0, and the arterial pH decreased progressively as the infusate pH was decreased below 3.0 (Gladden & Yates, 1983). Arterial PCO_2 remained very close to control conditions throughout all the infusions, averaging 34.3 to 36.0 mmHg, indicating that respiratory compensation was limited (Gladden & Yates, 1983). The conclusion of this study was that the pH of a NaLa/HLa infusion solution must remain above 3.4 and below 4.4 to eliminate significant blood pH changes when raising blood lactate to 10 mM (Gladden & Yates, 1983).

Miller et al (2005) conducted a lactate clamp study during rest and prolonged light to moderate-intensity exercise (55% $\dot{V}O_2$ peak) to analyze hematological and acid-base changes (Miller *et al.*, 2005). The NaLa/HLa mixture for infusion was prepared by mixing 88% (L+) lactic acid with 2 normal (N) NaOH, yielding a solution with a pH of 4.8 and a concentration entering the vein of 650 mM (Miller *et al.*, 2005). A pH of 4.8

was chosen due to the findings of Gladden and Yates that lactate infusates between the pH of 3.4 and 7.0 had the least effect on blood pH (Gladden & Yates, 1983). Miller et al. did not use the recommended range of a pH between 3.4 and 4.4 (Gladden & Yates, 1983) due to local irritation at the infusion site of human subjects. During rest and exercise, average lactate infusion rate was 0.032 and 0.037 $mmol \cdot kg^{-1} \cdot min^{-1}$, respectively (Miller *et al.*, 2005). Whole blood lactate concentration was elevated to 2.94 mM during rest for 90 min and then increased to a peak of 3.99 mM during 90 min of cycling exercise (Miller *et al.*, 2005). During rest, plasma PCO_2 was around 38 mmHg and increased to 41 mmHg during exercise (Miller *et al.*, 2005). Resting plasma HCO_3^- concentrations were about 26 mM; however, HCO_3^- concentrations increased significantly during exercise up to 32.9 mM (Miller *et al.*, 2005). Plasma pH increased from 7.44 to 7.46 during the 90 min of rest and from 7.46 to 7.53 during the 90 min of exercise (Miller *et al.*, 2005).

The study on NaLa/HLa infusion in humans by Miller et al. (2005) confirmed the findings by Gladden and Yates that an infusate pH above 4.4 will increase blood pH. Currently, NaLa/HLa infusion studies have only maintained infusion for short duration ($\leq$ 3 hours). In order for lactate infusion to be useful in the treatment of cancer, ventilator-induced lung injury, and TBI, a protocol of lactate infusion must be developed which allows maintenance of blood pH over an extended period of time at high lactate concentration ($\geq$8 mM). Before a protocol of this nature can ethically be used in humans, an animal model must be tested and deemed successful. Therefore, the purpose of this dissertation is to induce multi-hour hyperlactatemia, while maintaining a normal pH in the canine model.

II. JOURNAL MANUSCRIPT

ESTABLISHING PROLONGED HYPERLACTATEMIA IN A CANINE MODEL

ABSTRACT

The purpose of this study was to maintain acid-base and electrolyte balance, while inducing an eight-hour hyperlactatemic clamp of 8-9 mM using the canine model. Six adult dogs were anesthetized using sodium pentobarbital. Indwelling catheters were used for the infusion of 650 mM NaLa/HLa (pH 4.6), 80 mM KCl, and 42.5 mM $CaCl_2$. Arterial blood was also collected using indwelling catheters for the analysis of PO_2, PCO_2, pH, K^+, Na^+, Ca^{2+}, glucose, and lactate concentration. All animals were mechanically ventilated throughout the study. Ventilation was decreased to allow an increase in arterial PCO_2 for the maintenance of arterial pH, and oxygen was supplemented to the animals for the maintenance of normal arterial PO_2 levels. The pH of arterial blood fluctuated between 7.44 ± 0.02 and 7.48 ± 0.01 ($p > 0.05$). Sodium concentration in arterial blood rose from 142 ± 1 mM under control conditions to a max concentration of 154 ± 7 mM by nine hours of infusion ($p < 0.01$). Arterial K^+ and Ca^{2+} concentrations decreased slightly throughout the experiment from 3.3 ± 0.2 to 2.9 ± 0.4 mM and 1.28 ± 0.09 to 1.16 ± 0.19 mM, respectively; however, this decrease was not statistically significant ($p > 0.05$). This study successfully increased blood lactate concentration from 0.7 mM to approximately 8.0-9.0 mM over a period of eight hours.

Infusion of K^+ and Ca^{2+} with permissive hypercapnia and O_2 supplementation were required to maintain electrolyte and pH values within the physiological range.

INTRODUCTION

The earliest lactate infusion studies in humans were performed to study lactate metabolism. However, normal acid-base balance is difficult to maintain during lactic acid (HLa) or sodium lactate (NaLa) infusion. The reason acid-base balance is difficult to maintain during NaLa/HLa infusion is because lactic acid has two opposing effects on blood pH. It has an acidifying effect due to its dissociation properties as a weak acid (Schwartz & Waters III, 1962; Gladden & Yates, 1983) and its increase of the strong ion difference (SID) (Stewart, 1983); and an alkalinizing effect is caused by the oxidation of lactic acid to CO_2 and $H_2O + HCO_3^-$ or formation of glycogen + HCO_3^- and a concomitant decrease in the SID (Schwartz & Waters III, 1962; Gladden & Yates, 1983; Stewart, 1983). Therefore, any neutralization of a lactic acid infusate will diminish its acidifying effect and increase its alkalinizing effect (Gladden & Yates, 1983).

The early studies in humans demonstrated the difficulty of maintaining acid-base balance with NaLa infusion. Ahlborg et al. (1976) infused ten healthy male volunteers with NaLa (pH 7.0) for 30 min to raise blood lactate levels to 5 mM. Although blood pH was not measured, the authors acknowledged that an increase in pH likely took place due to NaLa infusion (Ahlborg *et al.*, 1976). Ryan et al. (1978) infused NaLa (pH 7.0) into 6 human volunteers for 20 minutes during rest and then 20 minutes during submaximal exercise to study the body's ability to metabolize lactate during rest and exercise. Plasma lactate levels in the subjects increased from a 1.3 mM baseline to 4.8 mM during NaLa infusion at rest and 9.1 mM by the end of exercise (Ryan *et al.*, 1979). Arterial blood pH

was measured, but not reported in this study. However, pH likely increased due to a decrease in SID with the metabolism of lactate and an increase of 1.9 mM HCO_3^- during resting infusion and 6.1 mM HCO_3^- during infusion under exercise conditions.

Chioléro et al. (1993) infused NaLa (pH 7.0) into six healthy human volunteers for a total of three hours. Plasma lactate levels were raised from 1.87 mM to 3.74 mM and pH increased from 7.38 to 7.47 by the end of the three hours (Chioléro *et al.*, 1993). Ferrannini et al. (1993) infused NaLa at a pH of 6.7 into an antecubital vein in 12 human subjects for three hours. Blood lactate concentrations rose from 0.80 mM to 2.91 mM, while blood pH also increased from 7.37 to 7.45 (Ferrannini *et al.*, 1993). Miller et al. conducted studies using La^- infusion to maintain a lactate clamp during rest and exercise in humans (Miller *et al.*, 2002a; Miller *et al.*, 2002b; Miller *et al.*, 2005). Results from these studies showed that La^- infusion at a pH of 4.8 can cause minor acid-base disturbances when blood lactate levels are raised from approximately 1 mM to approximately 5 mM (Miller *et al.*, 2002a; Miller *et al.*, 2002b; Miller *et al.*, 2005).

Due to the complexity of maintaining blood acid-base balance while infusing lactate, Gladden and Yates (1983) conducted a study in 14 anesthetized dogs to determine the optimal infusate pH to maintain normal blood acid-base balance. Lactate/lactic acid (NaLa/HLa) with pH varying from 2.0-7.0 at a concentration of 350-400 mM was infused for 60 min into the right brachial vein of the dogs (Gladden & Yates, 1983). Arterial blood lactate concentration increased from 1.4 mM during control conditions to 11.0 mM after 20 min of NaLa/HLa infusion, declining significantly to 9.8 mM after 30 min of infusion and then remaining constant for the final 30 min of infusion (Gladden & Yates, 1983).

Above an infusate pH of 4.4, the arterial pH increased by 0.118-0.167 during infusion; the arterial pH was essentially unchanged (0.00-0.056) when the infusate pH was between 3.4 and 4.0, and the arterial pH decreased progressively as the infusate pH was decreased below 3.0 (Gladden & Yates, 1983). The conclusion of this study was that the pH of a NaLa/HLa infusion solution must remain above 3.4 and below 4.4 to eliminate significant blood pH changes when raising blood lactate to approximately 10 mM via exogenous infusion.

Recently, hypoglycemia (low blood glucose) has been proposed as a potential therapy in the treatment of tumors exhibiting the Warburg effect (Nijsten & van Dam, 2009). The Warburg effect is a unique phenomenon in which cancer cells exhibit rapid glycolysis, termed aerobic glycolysis, despite an ample intracellular partial pressure of oxygen (iPO_2) for oxidative phosphorylation (Warburg, 1956; Vander Heiden *et al.*, 2009). Hypoglycemia, however, can greatly impair the function of an organism if a salvage fuel cannot be provided in place of glucose.

The release of lactate from one cell and its uptake by another cell occurs through monocarboxylate transport proteins (MCTs) in a process called the cell-cell lactate shuttle (Brooks, 1998). MCTs have been found in most mammalian tissue including the heart, brain , and skeletal muscle (Pellerin *et al.*, 1998; Price *et al.*, 1998; Hashimoto *et al.*, 2008; Brooks, 2009). Therefore, lactate has been proposed as a proper replacement fuel since it can be oxidized by the vast majority of healthy cells, but not very well in cancer cells exhibiting the Warburg effect (Nijsten & van Dam, 2009). Therefore, it is possible that inducing hypoglycemia, while infusing NaLa will allow normal cells to

survive, while killing tumor cells or making the tumor cells more succesptible to conventional treatments such as radiation and chemotherapy.

The infusion of lactate may also be beneficial to patients who have sustained traumatic brain injury (TBI). Research has shown that the brain may preferentially metabolize lactate in response to TBI as indicated by increased cerebrospinal fluid (CSF) and extracellular fluid (ECF) lactate concentrations in the injured brain following trauma (King *et al*., 1974; Inao *et al.*, 1988). This increased level of lactate in the brain may be beneficial as studies have reported that lactate primarily fuels recovery of synaptic function after hypoxia upon reoxygenation (Schurr *et al.*, 1988; Schurr *et al.*, 1997) and that increasing brain lactate levels results in better recovery than if brain glucose levels are increased in the rat following fluid percussion injury (FPI) (Chen *et al.*, 2000a; Rice *et al.*, 2002). In any case, further study on the effects of acid-base balance from lactate infusion is required before the use of lactate infusion as a treatment can be put into practice.

The results of previous lactate infusion studies indicate that it is a challenge to maintain normal acid-base balance, while clamping blood lactate at a high level (8 or more mM) over the course of several hours. If the infusate pH is too low, blood pH will decline rapidly, hemolysis can occur, and death may result. If the infusate pH is in the neutral range, the blood pH will rise to alkalotic levels as a portion of the infused lactate is oxidized. Also, prior studies of lactate infusion have made little note of electrolyte changes. Currently, there are no data on hyperlactatemia due to NaLa/HLa infusion for greater than three hours. Therefore, the purpose of the current study was to maintain acid-balance at control, physiological conditions during a "hyperlactatemic clamp" with a

blood lactate concentration of about 8-9 mM for eight hours in anesthetized dogs, while maintaining stable blood gases, acid-base status, and electrolyte balance.

METHODS AND PROCEDURES

Animals. All experimental procedures performed in this study were approved by the Auburn University Institutional Care and Use Committee (Auburn, AL, USA). Six adult mongrel hounds (*Canis lupus familiaris*) of both sexes were used (15.0-20.4 kg). Canines were used in this study because their physiology is similar to humans and the blood volume of the average canine is sufficient for serial blood sampling. The dogs were housed at the Division of Laboratory Animal Health facility at the Auburn University Veterinary School, with access to food and water *ad libitum*. All animals were fasted from food 48 hours prior to experimental use.

Animal preparation. On each experimental day, the animals were anesthetized by injection of sodium pentobarbital (30 $mg \cdot kg^{-1}$ body weight) into a prominent cephalic vein of the forelimb. This established a deep surgical plane of anesthesia which was maintained throughout the experiment with additional doses of sodium pentobarbital given as necessary into an isolated jugular vein to maintain absence of pedal, palpebral, and corneal reflexes. An endotracheal tube was inserted into the trachea and the dogs were ventilated with a mechanical respirator (Harvard Apparatus, model 613, Holliston, MA). The initial respirator settings consisted of a 20 $ml \cdot kg^{-1}$ body weight tidal volume and a breathing frequency of 15-20 breaths/min; however, ventilation was subsequently adjusted throughout to minimize increases in arterial pH, if possible. Rectal temperature was maintained at approximately 37˚C throughout the experiment using a commercial heating pad.

Surgical preparation. Femoral arteries and veins were exposed by an incision near the inguinal notch. Tissues overlaying the femoral vein and artery were cut with a cauterizing blade (electric soldering gun). The right femoral artery was cannulated and a blood pressure transducer (model RP-1500, Narco Biosystems) inserted for measurement of systemic arterial pressure. In all experiments indwelling catheters were used for infusion and sampling. One femoral artery was used for the measurement of blood pressure and sampling, while the other femoral vein was used for infusion of NaLa and potassium chloride (KCl). One external jugular vein was used for infusion of calcium chloride ($CaCl_2$), and the other was used for sampling. A Foley catheter was inserted into the urethra for urine collection. Three separate peristaltic pumps (Gilson Minipuls 3, Gilson, Inc., Middleton, WI USA) were used for infusion of NaLa, KCl, and $CaCl_2$. All infusion lines were flushed with sodium heparin (15 U/mL) to prevent clotting of the lines between sampling periods. At the end of the experiment the animals were euthanized with an overdose of sodium pentobarbital and saturated KCl while still under anesthesia (AVMA, 2001).

Experimental design. A 650 mM NaLa/HLa infusion solution at a pH of 4.6 was made by combining 85 percent NaLa with 15 percent HLa. This solution was infused into a femoral vein to raise arterial blood lactate levels from a normal concentration of about 1 mM to 10 mM. The first blood sample was drawn three minutes after the start of infusion, the second sample was drawn 6 minutes after the start of infusion, and the third sample was drawn 87 minutes after the start of infusion to ensure that all blood-gas levels were in the normal physiological range and that blood lactate levels reached 8 mM. After 87 minutes of infusion blood lactate reached 8 mM and blood samples were then taken at

approximately 60-minute intervals. Blood samples were drawn from designated catheters and analyzed for arterial partial pressure of oxygen (PO_2), partial pressure of carbon dioxide (PCO_2), pH, potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), glucose, and lactate concentrations using a blood gas, pH analyzer (IL GEM Premier 3000). Arterial concentration of bicarbonate (HCO_3^-) and base excess (BE) were calculated using the Henderson-Hasselbalch (Po & Senozan, 2001) and Siggaard-Andersen (Siggaard-Andersen, 1971) equations, respectively. When a trend towards higher blood pH was observed, ventilation was reduced to allow PCO_2 to rise in order to minimize blood pH increases above 7.4. Oxygen was administered during ventilation to maintain a normal arterial partial pressure of oxygen (PO_2) at approximately 100 mmHg. When changes in arterial K^+ or Ca^{2+} concentration were observed, KCl (80 mM) or $CaCl_2$ (42.5 mM) were infused as necessary to maintain normal blood levels. Sodium lactate infusion was also varied during the study to maintain blood lactate concentrations of at least 8 mM.

Statistical analysis. Data are presented as means ± standard deviations. One-way repeated measures analysis of variance (RMANOVA) with the repeated measures occurring across time was used to compare means. A Greenhouse-Geisser correction was used when the assumption of sphericity was not met (Muller & Barton, 1989). Bonferroni pairwise comparisons were used when significant differences were present among protocols. Alpha was set at 0.05, and all statistics were generated using OriginPro 8.1 (OriginLab Corporation, Northampton, MA USA).

RESULTS

Throughout each experiment, the infusion rates of NaLa/HLa, KCl, and $CaCl_2$ were varied to cause hyperlactatemia while maintaining arterial concentrations of Na^+,

K^+, and Ca^{2+} within physiological range if, possible. Infusion of NaLa began at a rate of approximately 0.13 $mmol \cdot kg^{-1} \cdot min^{-1}$ for the first 10 minutes of infusion and then decreased to a range of 0.10-0.09 $mmol \cdot kg^{-1} \cdot min^{-1}$ after 90 minutes of infusion (Figure 1). Within 43 minutes of the study, KCl infusion was required. The rate of KCl infusion began 0.002 $mmol \cdot kg^{-1} \cdot min^{-1}$ and then ranged from 0.004 - 0.008 $mmol \cdot kg^{-1} \cdot min^{-1}$ over the remaining seven hours (Figure 2). Infusion of $CaCl_2$ (Figure 3) began 43 minutes after commencement of the study. The rate of $CaCl_2$ infusion started at 0.001 $mmol \cdot kg^{-1} \cdot min^{-1}$ and steadily increase over seven hours where the infusion rate plateaued at about 0.002 $mmol \cdot kg^{-1} \cdot min^{-1}$.

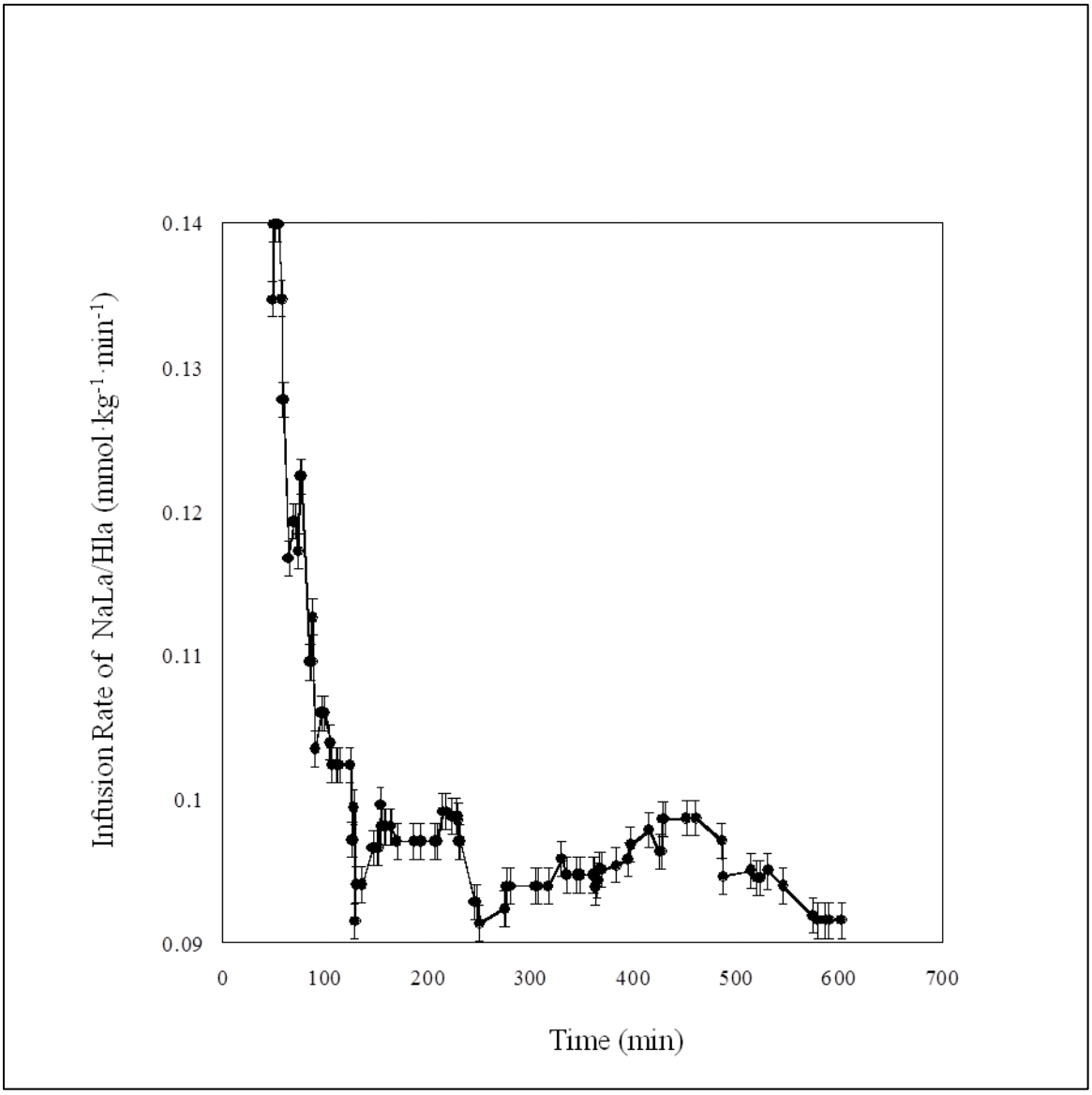

Figure 1: Mean±SD infusion rate of NaLa/HLa ($mmol \cdot kg^{-1} \cdot min^{-1}$) across all six dogs.

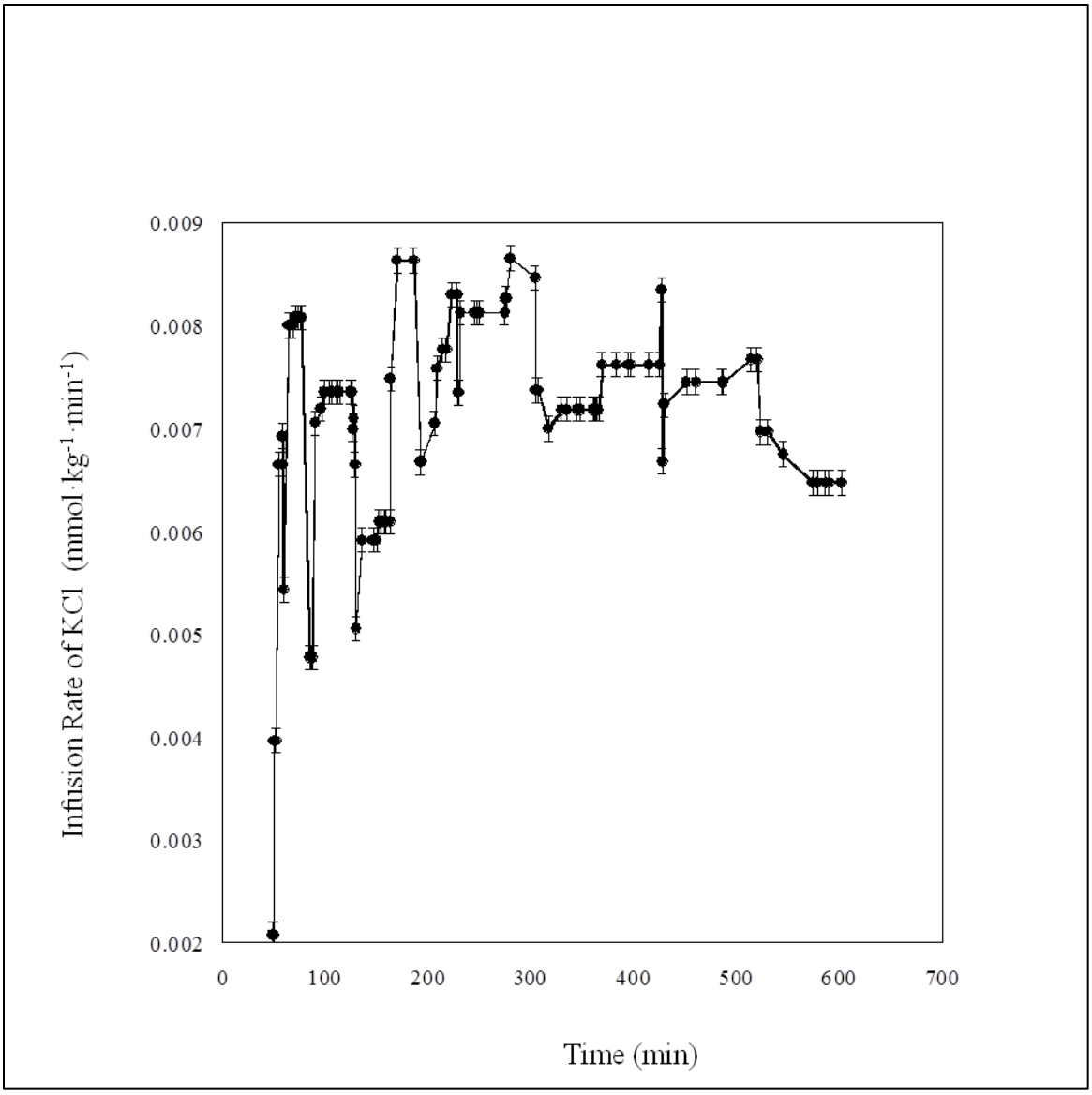

Figure 2: Mean±SD Average infusion rate of KCl ($mmol \cdot kg^{-1} \cdot min^{-1}$) across all six dogs.

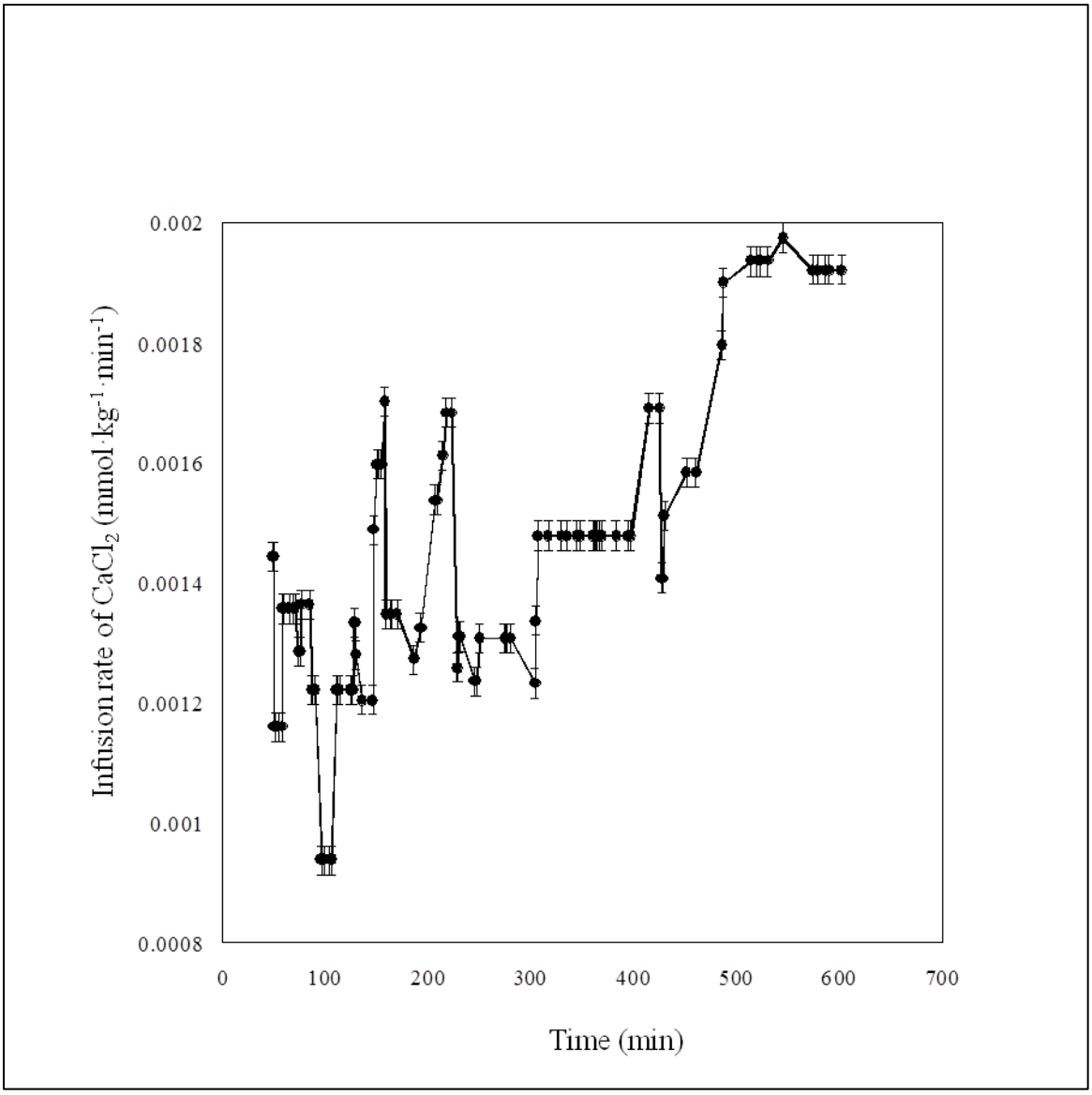

Figure 3: Mean±SD infusion rate of $CaCl_2$ (mmol·kg^{-1}·min^{-1}) across all six dogs.

All arterial blood lactate, pH, PCO_2, [HCO_3^-] and BE levels over the ten hour experiments are presented in table 1. Arterial blood lactate concentration increased significantly from 0.65 ± 0.44 mM under control conditions to 8.0± 0.8 mM ($P < 0.01$) after eight hours of Na/HLa infusion (Figure 4). The concentration of arterial blood lactate then decreased slightly to 8.8 ± 0.8 mM after nine hours and then rose again to 8.8 ± 0.7 mM after ten hours. The pH of arterial blood fluctuated between 7.44 ± 0.02 and

7.48 ± 0.01 ($p > 0.05$) throughout the ten hour experiment (Figure 5). Figure 6 shows that the reduction of ventilation to maintain arterial pH caused the PCO_2 in arterial blood to significantly increase from 30 ± 5 mmHg at rest to 59 ± 5 mmHg at the end of the ten hour experiment ($p < 0.01$). As expected with lactate infusion, arterial [HCO_3^-] increased significantly from 20.9 ± 1.5 mM under control conditions to 39.1 ± 3.5 mM after ten hours of NaLa/HLa infusion ($p < 0.01$) (Figure 7). In conjunction with HCO_3^- concentration, BE also increased steadily from -2.22 ± 1.40 mM under control conditions to 14.23 ± 3.28 mM after ten hours of NaLa/HLa infusion ($p < 0.01$) (Figure 8).

Table 1: Mean±SD for arterial blood levels of lactate, pH, PCO_2, HCO_3^-, and BE.
* = values statistically different compared to 3 and 6 minutes.
† = values statistically different compared to 87 minutes.
‡ = values statistically different compared to 123 minutes.
! = values statistically different compared to 184 minutes.

Time (min)	**Lactate (mM)**	**pH**	**PCO_2 (mmHg)**	**HCO_3^- (mM)**	**BE (mM)**
3	0.7±0.4	7.47±0.06	30±5	20.9±1.5	-2.22±1.40
6	0.7±0.4	7.46±0.05	30±5	20.7±1.5	-2.63±1.38
87	8.8±1.3*	7.47±0.02	38±5	26.6±2.1*	2.92±1.86*
123	8.6±1.3*	7.47±0.03	42±5*	29.8±3.2*	5.96±3.09*
184	8.6±0.5*	7.46±0.04	50±7*†	33.9±2.4*†‡	9.64±2.11*†‡
238	8.8±0.7*	7.46±0.05	54±6*†‡	36.7±1.8*†‡	12.23±1.84*†‡
302	8.3±0.5*	7.46±0.04	56±8*†‡	37.9±2.9*†‡!	13.36±2.44*†‡!
363	8.2±0.8*	7.48±0.01	53±5.4*†‡	37.5±3.1*†‡	13.24±2.74*†‡!
424	8.5±0.9*	7.47±0.03	55±4*†‡	38.2±2.9*†‡!	13.73±2.92*†‡!
482	9.0±0.8*	7.45±0.03	58±5*†‡	38.9±3.8*†‡!	14.24±3.60*†‡!
543	8.8±0.8*	7.45±0.02	59±6*†‡	38.8±4.2*†‡!	14.05±3.92*†‡!
602	8.8±0.7*	7.44±0.02	59±5*†‡	39.0±3.5*†‡!	14.23±3.28*†‡!

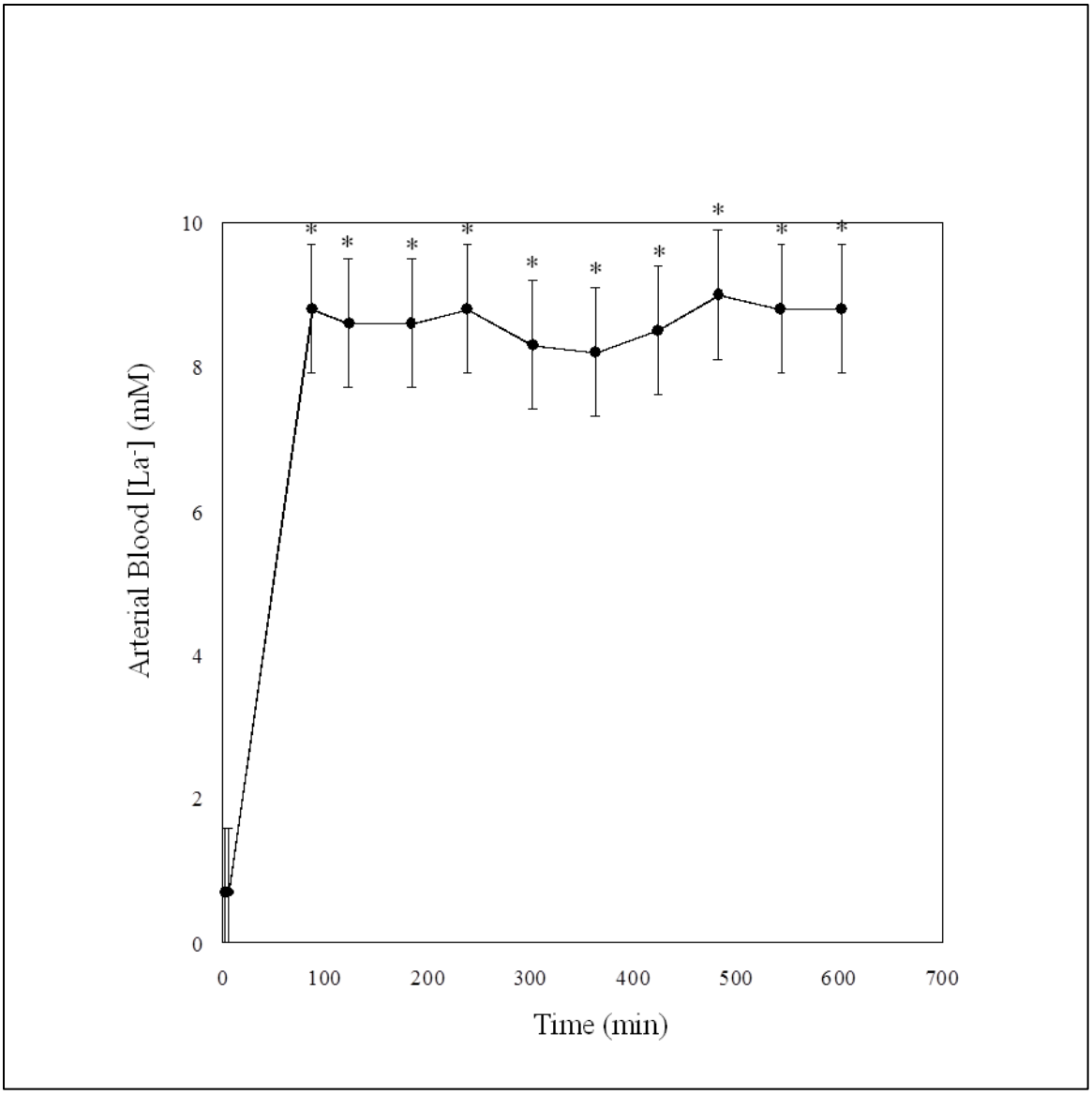

Figure 4: Mean±SD arterial blood lactate concentration across all six dogs.
* = values statistically different compared to 3 and 6 minutes.

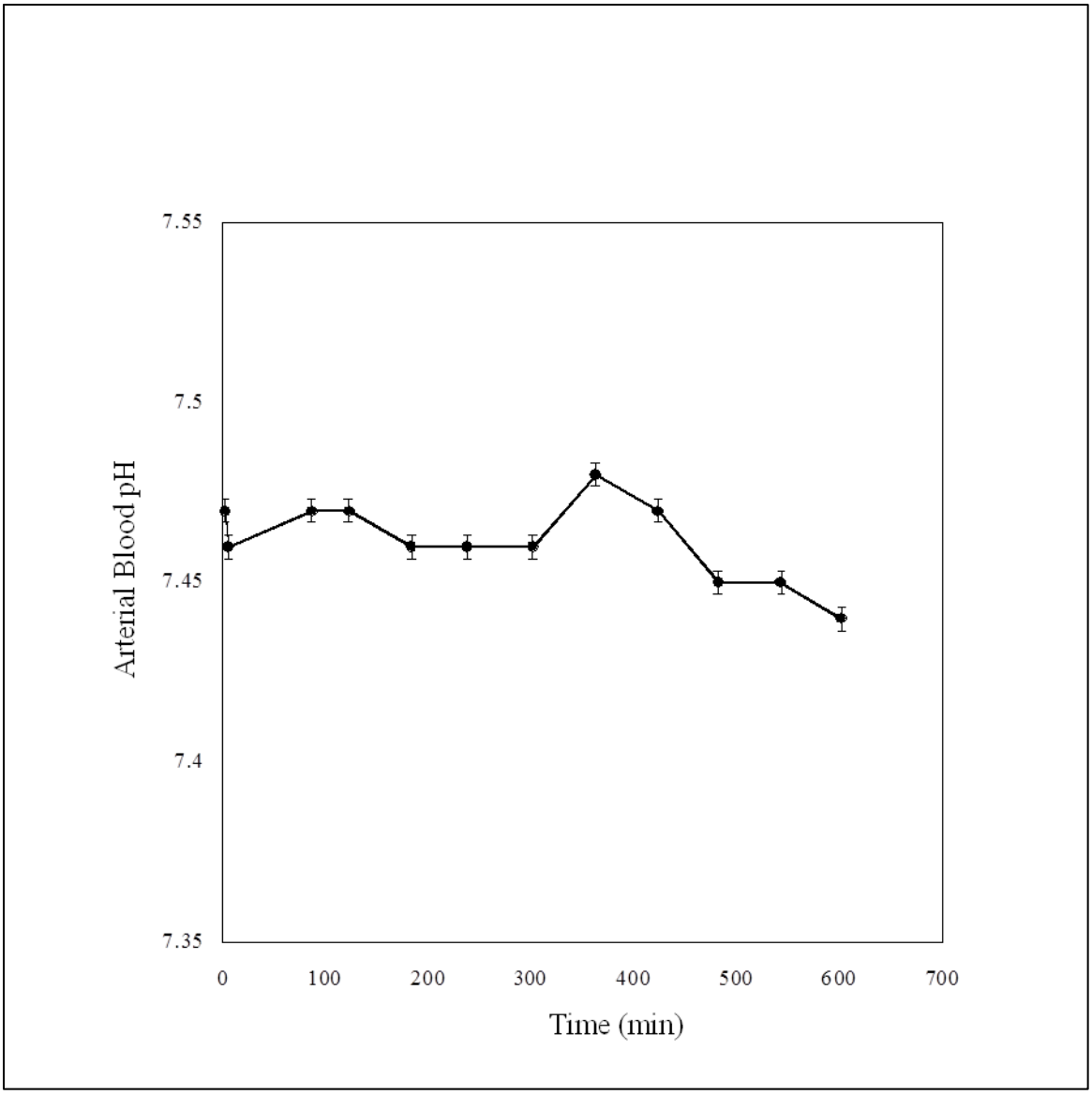

Figure 5: Mean±SD arterial pH across all six dogs.

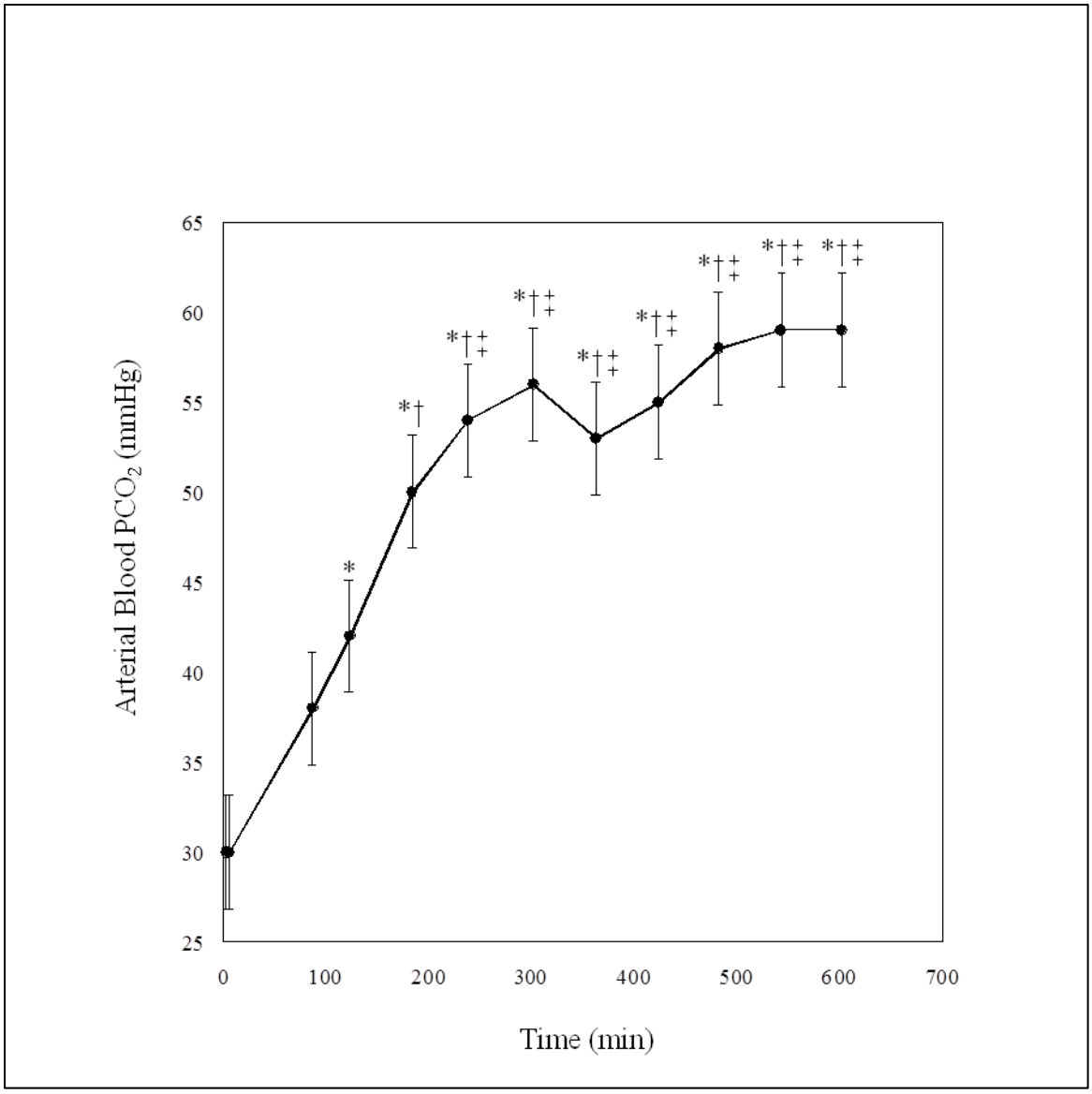

Figure 6: Average arterial blood PCO_2 with permissive hypercapnia across all six dogs.
* = values statistically different compared to 3 and 6 minutes.
† = values statistically different compared to 87 minutes.
‡ = values statistically different compared to 123 minutes.

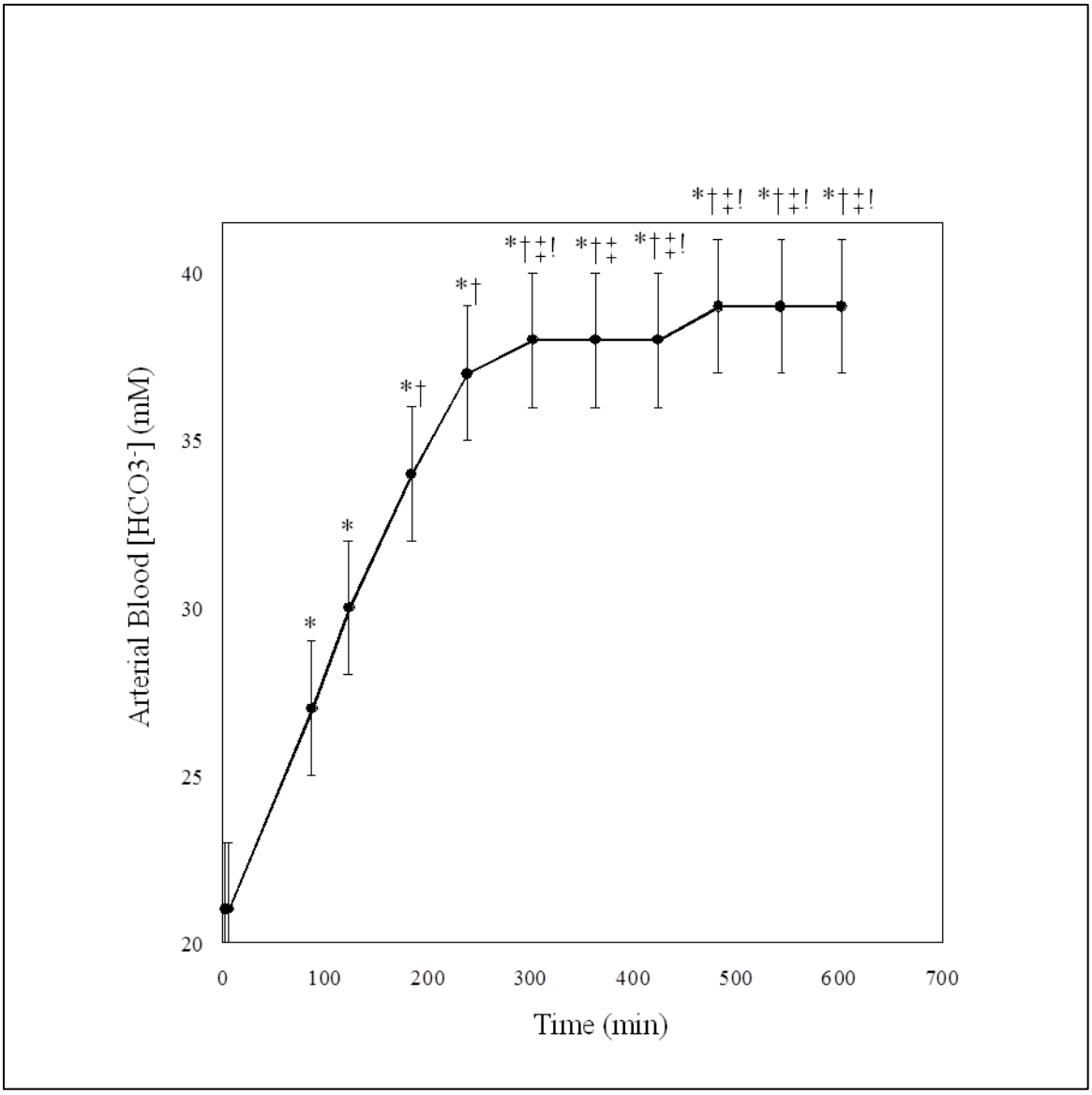

Figure 7: Mean±SD arterial blood bicarbonate concentration across all six dogs.
* = values statistically different compared to 3 and 6 minutes.
† = values statistically different compared to 87 minutes.
‡ = values statistically different compared to 123 minutes.
! = values statistically different compared to 184 minutes.

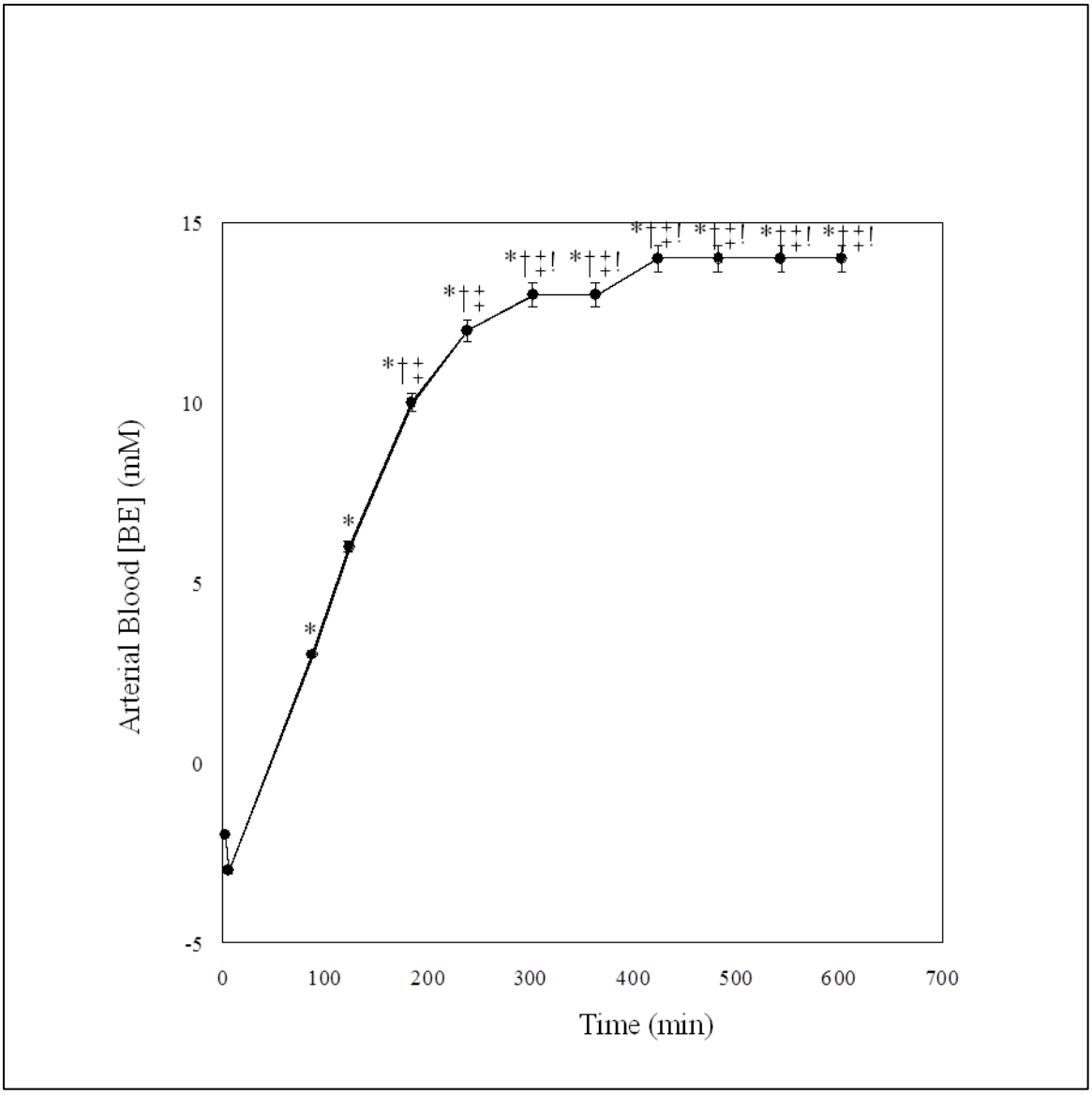

Figure 8: Mean±SD arterial blood base excess concentration across all six dogs.
* = values statistically different compared to 3 and 6 minutes.
† = values statistically different compared to 87 minutes.
‡ = values statistically different compared to 123 minutes.
! = values statistically different compared to 184 minutes.

All arterial blood PO_2, glucose, Na^+, K^+, and Ca^{2+} levels over the ten hour experiments are presented in table 2. Arterial PO_2 fluctuated between 96 ± 11 mmHg and 129 ± 19 mmHg throughout the ten hour experiment ($p > 0.05$) (Figure 9). The arterial concentration of glucose fluctuated between 5.4 ± 0.2 mM and 6.0 ± 0.6 mM throughout the experiment ($p > 0.05$) (Figure 10). Sodium (Na^+) concentration in arterial blood rose

significantly from 142 ± 1 mM under control conditions to a max concentration of 154 ± 7 mM by nine hours of infusion ($p < 0.01$) (Figure 11). Arterial postassium (K^+) and calcium (Ca^{2+}) concentrations decreased slightly throughout the experiment from 3.3 ± 0.2 mM to 2.9 ± 0.4 mM ($p > 0.05$) and from 1.28 ± 0.09 mM to 1.16 ± 0.19 mM ($p > 0.05$), respectively (Figure 12 and 13). The total volume of urine collected throughout the experiment was 1.91± 0.92 L.

Table 1: Mean±SD for arterial blood levels of lactate, pH, PCO_2, HCO_3^-, and BE.
* = values statistically different compared to 3 minutes.
† = values statistically different compared to 6 minutes.

Time (min)	**PO_2 (mmHg)**	**Glucose (mM)**	**Na^+ (mM)**	**K^+ (mM)**	**Ca^{2+} (mM)**
3	98±13	6±0	142±1	3.3±0.2	1.28±0.09
6	96±10	5±0	143±1	3.3±0.3	1.27±0.06
87	114±17	6±1	147±3	3.0±0.3	1.18±0.10
123	106±14	6±1	149±4*	3.3±0.7	1.17±0.14
184	125±30	6±1	150±3*†	3.1±0.2	1.18±0.17
238	129±19	6±0	152±4*†	3.1±0.1	1.18±0.17
302	115±10	6±0	152±6*†	3.2±0.4	1.19±0.18
363	122±15	6±0	153±5*†	3.0±0.2	1.17±0.16
424	124±15	6±0	153±5*†	3.1±0.2	1.17±0.16
482	117±16	6±0	153±6*†	3.1±0.2	1.15±0.15
543	115±11	6±0	154±7*†	3.2±0.3	1.18±0.17
602	112±17	6±1	154±7*†	2.9±0.4	1.16±0.19

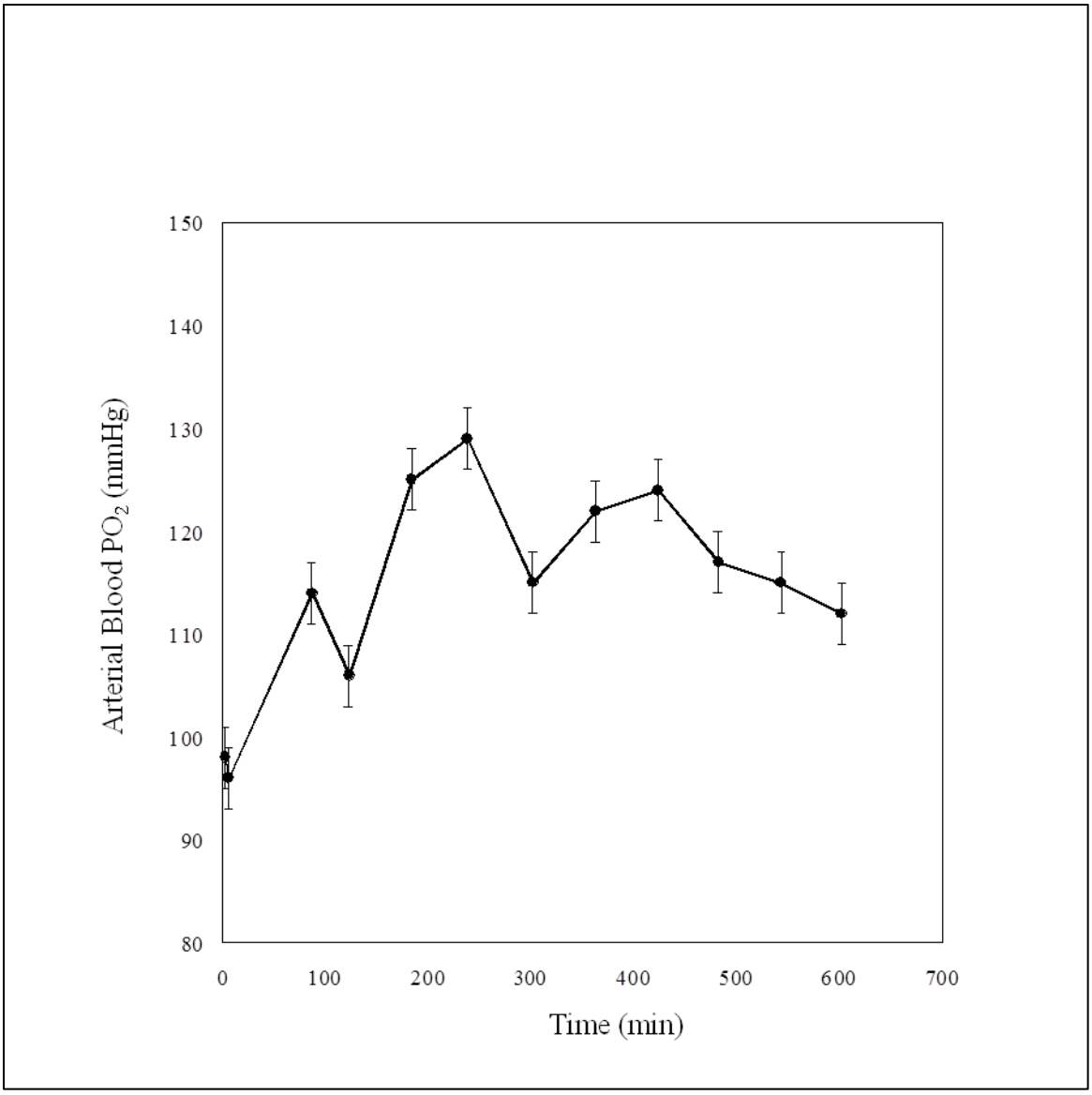

Figure 9: Mean±SD arterial blood PO_2 across all six dogs.

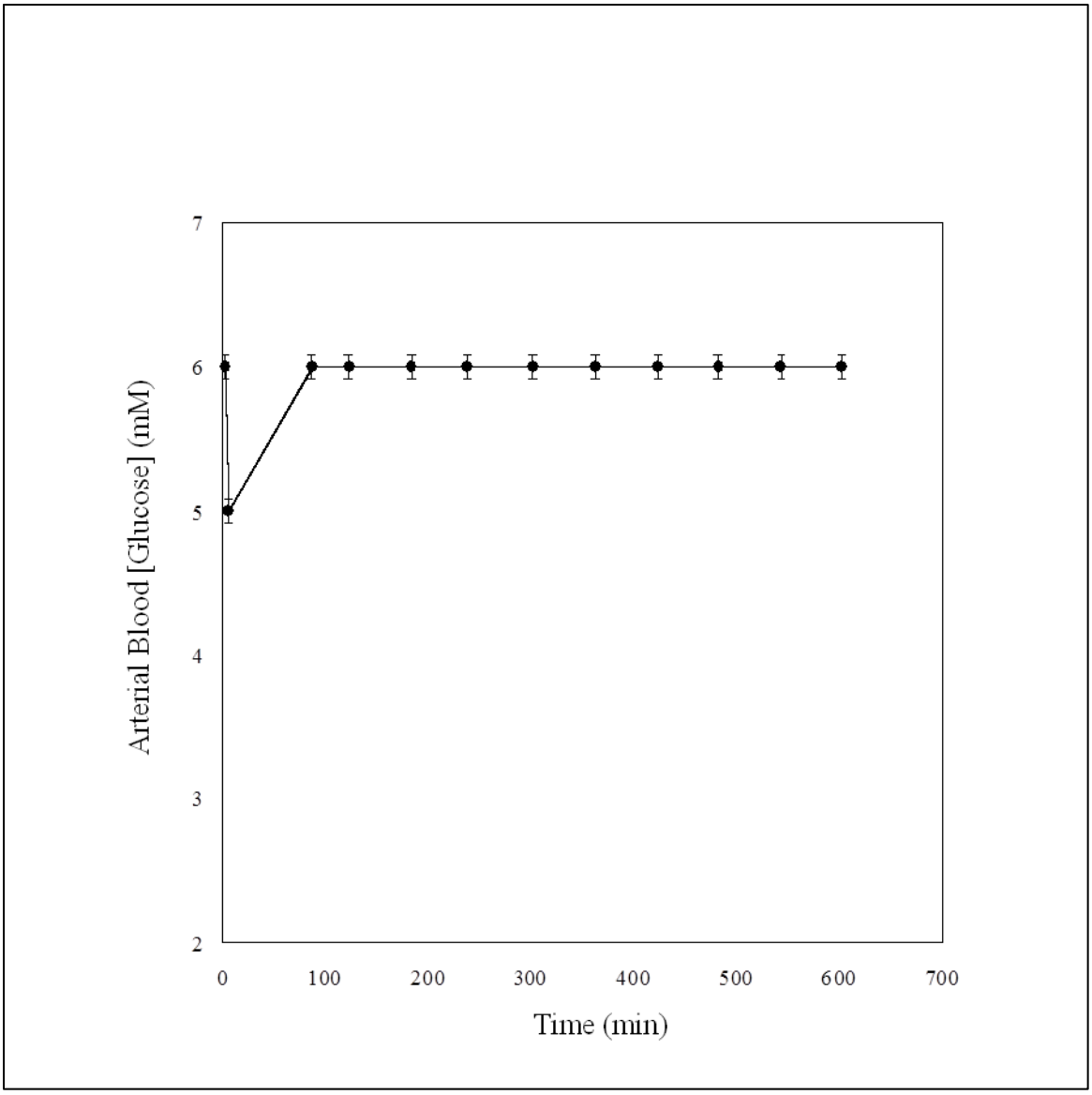

Figure 10: Mean±SD arterial blood glucose concentration across all six dogs.

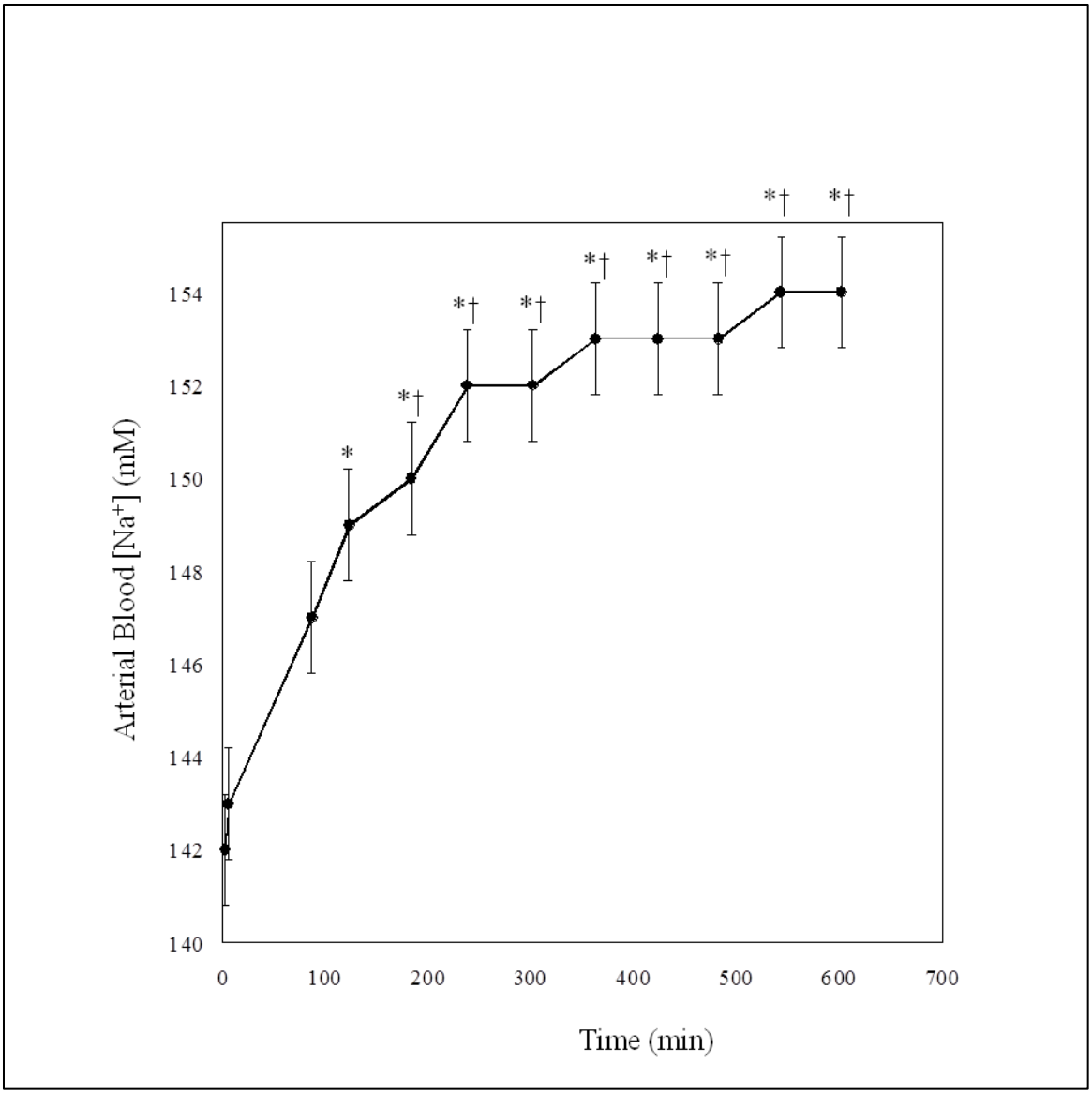

Figure 11: Mean±SD arterial blood sodium concentration across all six dogs.
* = values statistically different compared to 3 minutes.
† = values statistically different compared to 6 minutes.

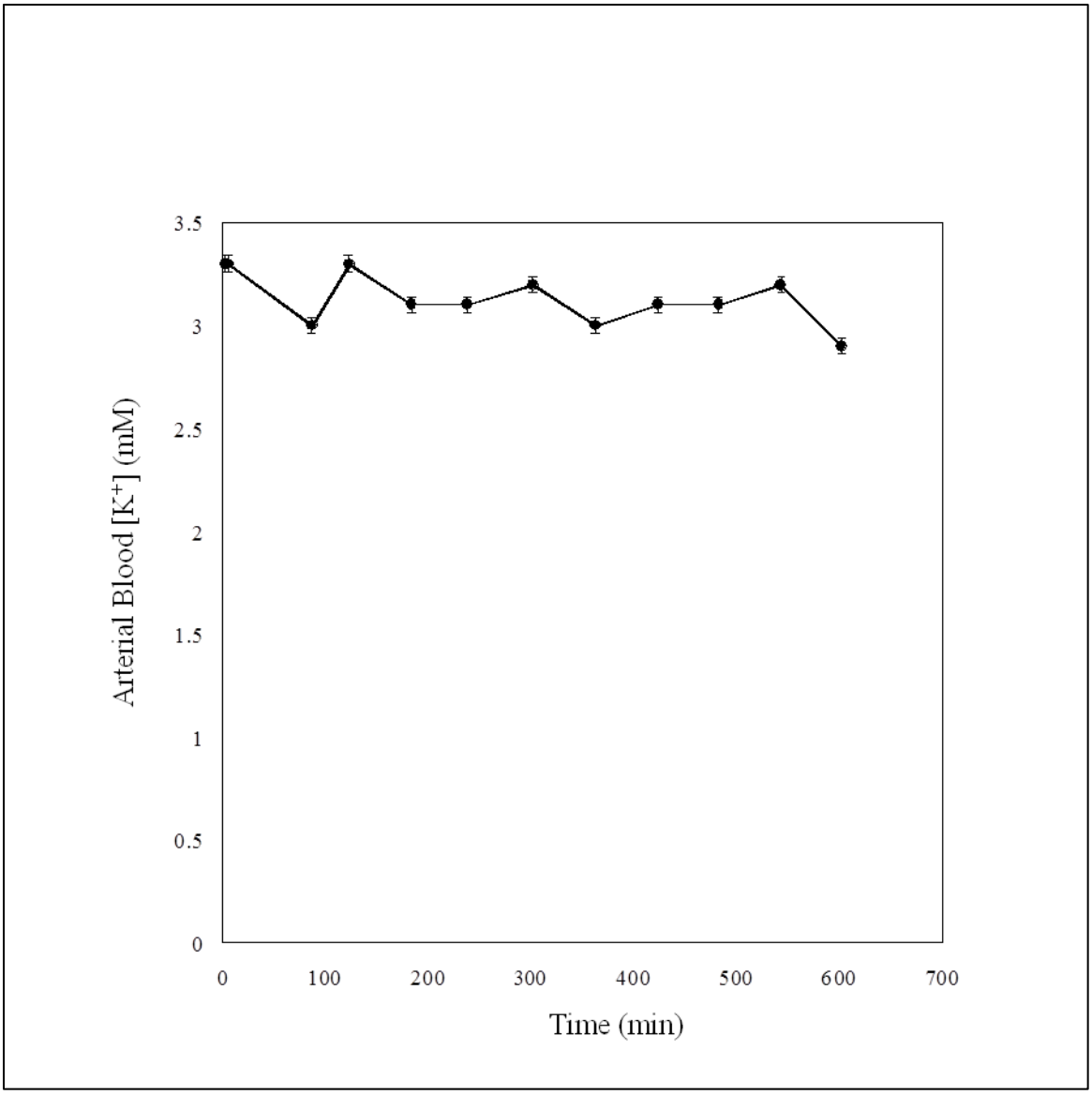

Figure 12: Mean±SD arterial blood potassium concentration across all six dogs.

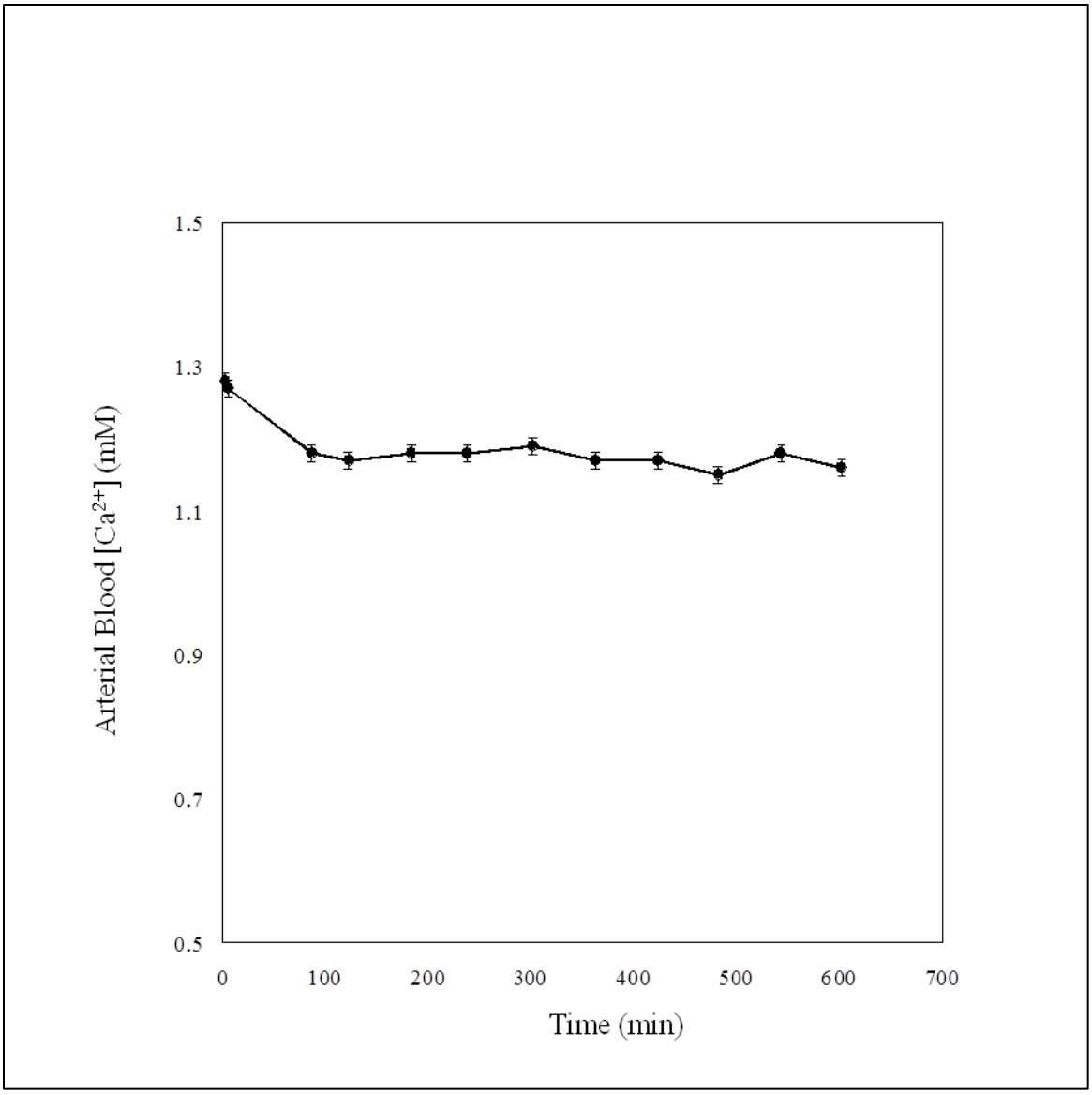

Figure 13: Mean±SD arterial blood calcium concentration across all six dogs.

DISCUSSION

The primary finding of this study is that infusion of a 650 mM NaLa/HLa solution with a pH of 4.6 can induce a hyperlactatemic clamp of 8-9 mM for a period of eight hours without causing significant acid-base changes. A pH of 4.6 was chosen because it was within the range suggested by Gladden and Yates (1983) to maintain physiological acid-base balance, while increasing blood lactate concentration to 10 mM.

Infusion of NaLa/HLa did cause a significant increase in HCO_3^- and BE arterial concentrations. This was expected since the metabolism of lactate to CO_2 and H_2O along with the synthesis of lactate to glycogen both cause the formation of HCO_3^- anions (Schwartz & Waters III, 1962; Gladden & Yates, 1983) and a decrease in SID (Stewart, 1983), causing an increase in blood pH. To counteract the effect of HCO_3^- on blood pH, ventilation was decreased allowing a permissive and significant rise in arterial PCO_2 (See Figure 6). Oxygen was supplemented throughout the study and maintained normal arterial PO_2 levels (See Figure 9).

Glucose levels were not affected during NaLa/HLa infusion, which is a significant finding since lactate has been shown to decrease free fatty acid (FFA) metabolism (Issekutz & Miller, 1962). With a decrease in FFA metabolism, blood glucose levels would be expected to decrease. However, this finding supports previous research showing that not only can lactate be oxidized by cells in preference to glucose (Brooks, 2000), but it is also a potent substrate for gluconeogenesis by the liver via the Cori cycle (Cori, 1931). Therefore, when blood lactate concentration is high, blood glucose levels are maintained due to the use of lactate as a fuel by cells along with the liver using lactate as a substrate for gluconeogensis and the introduction of new glucose into the blood.

The success of creating a hyperlactatemic clamp while maintaining normal acid-base and electrolyte balance in the canine model is an important step in the possibility of using lactate as a salvage fuel in the treatment of cancer cells with hypoglycemia, termed lactate protected hypoglycemia (LPH) (Nijsten & van Dam, 2009). Withdrawing glucose from cancer cells has been shown to induce cell death *in vitro* (Vander Heiden *et al.*, 2001); however, this treatment would be lethal *in vivo* unless a salvage fuel was infused

to protect healthy cells without that salvage fuel also supplementing the cancer cells. Glycerol has been tested as a salvage fuel during hypoglycemia in rats and one cancer patient (Burt *et al.*, 1985). In that study gluconeogenesis was inhibited with the drug 3-mercaptopicolinate (3MP), which inhibits the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) (Burt *et al.*, 1985); however infused glycerol enters the gluconeogenic pathway distal to PEPCK and therefore is not likely a good treatment for maintaining hypoglycemia (McCarty, 2001).

Lactate is a candidate for use as a salvage fuel, since it has been demonstrated that most healthy cells can metabolize lactate as an energy source (Pellerin *et al.*, 1998; Price *et al.*, 1998; Gladden, 2004a, b; Hashimoto *et al.*, 2008; Brooks, 2009) and has also been shown to assist in the maintenance of hypoglycemia (Maran *et al.*, 1994). Cancer cells exhibiting the Warburg effect may be especially susceptible to LPH treatment, since they are net lactate producers, and consume copious amounts of glucose (Nijsten & van Dam, 2009). The obvious next step in verifying lactate as an appropriate salvage fuel during hypoglycemia is to induce a multi-hour LPH study in the canine model to determine if life can be maintained under conditions of low glucose, and if low blood glucose levels result in significant tissue and organ damage.

The ability to raise arterial blood lactate levels as shown in this study may also be useful for the treatment of TBI in the clinical setting since the injured brain has been shown to preferentially metabolize lactate over glucose (King *et al.*, 1974; Inao *et al.*, 1988; Chen *et al.*, 2000a; Chen *et al.*, 2000b). Traumatic brain injury is a multifaceted process which includes ischemia, high intracellular calcium levels, and impaired mitochondrial function (Rice *et al.*, 2002). Supplying the brain with intravenous lactate

after a TBI may aid in the recovery and prevention of permanent brain damage since lactate has been shown to be the primary fuel used in the recovery of synaptic function after brain hypoxia (Schurr *et al.*, 1988; Schurr *et al.*, 1997). Infusion of lactate early after TBI may benefit the injured brain by improving Krebs Cycle metabolism, restoring ionic homeostasis, and preserving glucose for later use in recovery (Chen *et al.*, 2000b). Rice et al. (2002), conducted a study on rats following lateral fluid percussion injury (FPI) to the hippocampus of the brain and found that lactate infusion during recovery resulted in better memory and learning compared to saline infused rats (Rice *et al.*, 2002). The findings of our study show that lactate infusion can be done successfully in dogs without acid-base and electrolyte imbalance, thereby providing a potential platform to utilize lactate infusion for the treatment of TBI in humans.

Lactic acid infusion may also benefit people with acute respiratory distress syndrome (ARDS), which results in an increased ventilatory dead space impairing the clearance of CO_2 from the blood (Nuckton *et al.*, 2002). In order to maintain physiological blood pH and PCO_2, patients with ARDS must be ventilated mechanically to increase minute ventilation (De Campos, 2000); however, mechanical ventilation has been shown to cause lung injury (Mascheroni *et al.*, 1988). In order to prevent damage from mechanical ventilation, extracorporeal carbon dioxide removal ($ECCO_2R$) has been implemented with the use of a membrane lung (ML) (Terragni *et al.*, 2009; Bein *et al.*, 2013). Unfortunately, extracorporeal blood flow must be in the range of 0.5 to 1 L/min rendering this technique useful only in major medical centers and severely ill patients (Zanella *et al.*, 2014). The infusion of lactic acid has been proposed for use in $ECCO_2R$ to assist in CO_2 removal by converting bicarbonate ions into CO_2 thereby increasing the

PCO_2 transmembrane gradient and subsequent CO_2 transfer (Zanella *et al.*, 2009). An increase in PCO_2 may allow a lower blood flow thereby making $ECCO_2R$ a more widespread and effective treatment in the prevention of mechanical ventilator induced lung injury. Thus far only extracorporeal lactic acid infusion using a ML in swine has been successful at removing excess CO_2 (Zanella *et al.*, 2014). Future studies should be conducted to assess how HLa infusion can assist in $ECCO_2R$ without causing severe acidosis.

Another purpose of this study was to maintain electrolyte balance of Na^+, K^+, and Ca^{2+} throughout the eight hour hyperlactatemic clamp, since it has been shown that these ions fluctuate when canines are anesthetized (Sudzhyan, 1963). Sodium levels increased significantly throughout the study period, but remained within the normal range of Na^+ in conscious dogs (Nielsen *et al.*, 2010). The concomitant infusion of Na^+ with lactate likely caused the increase in arterial sodium levels, since it has been found that pentobarbital anesthesia can cause natriuresis due to partial inhibition of the tubular reabsorptive process for sodium (Blake, 1957), so that if Na^+ was not being infused with lactate the arterial concentration of Na^+ would have likely decreased throughout the study period.

Both arterial K^+ and Ca^{2+} levels were slightly below the normal range for concious dogs throughout the experiment (Nielsen *et al.*, 2010); however, this response to barbituate anesthesia has been shown previously using thiopental (Sudzhyan, 1963). Although K^+ and Ca^{2+} levels decreased, the infusion of KCl and $CaCl_2$ prevented a drop in K^+ and Ca^{2+} that was statistically significant. As to the extent of our knowledge K^+ and Ca^{2+} arterial concentrations have not previously been monitored during anesthesia

specifically with pentobarbital, and it is therefore unknown as to why these cations tended to decrease during the experiment. Perhaps renal excretion of K^+ and Ca^{2+} occurred; however the concentration of K^+, and Ca^{2+} in urine was not analyzed in this study.

In conclusion, this study is the first to establish a hyperlactatemic clamp (≈9 mM) for eight hours while maintaining acid-base and electrolyte balance. Results from this study indicate that arterial lactate levels can be raised over a period of hours in the anesthetized canine without adverse effects or death of the animal. This is an important finding because as of late there have been suggestions from the clinical community that lactate infusion may be a viable treatment for cancer cells exhibiting the Warburg effect, patients with TBI, and patients with ARDS who need to be mechanically ventilated. Significant information from the current study is that permissive hypercapnia along with infusion of KCl and $CaCl_2$ are key elements in maintaining otherwise physiologically normal blood electrolyte and pH profiles during significant hyperlactatemia.

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