Serial plasma lactate concentrations in 68 puppies aged 4 to 80 days

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Abstract

Objective: To determine a reference range for venous blood lactate concentrations in healthy neonatal dogs.

Design: A prospective cohort study.

Setting: All work was conducted at the College of Veterinary Medicine, Texas A&M University.

Animals: Clinically healthy dogs: 68 puppies and 30 adults.

Measurements and main results: A blood sample was collected from each puppy into lithium heparin via jugular venipuncture at 4, 10, 16, 28, 70, and 80 days of age. A single venous sample was collected from each adult dog. Lactate concentration in each sample was measured immediately using an automated analyzer. Two hundred seventy-seven blood samples were analyzed. Blood lactate concentrations of adult dogs were 1.80 ± 0.84 mmol/L (mean ± SD). Mean blood lactate concentrations of puppies were significantly higher at 4, 10, 16, and 28 days of age compared with those of adult dogs. The reference range for lactate concentration for puppies at 4 days of age was 1.07–6.59, and for the puppies from 10 to 28 days of age was 0.80–4.60.

Conclusions: Assessment of perfusion can be challenging in neonates due to normal physiologic variation and small size. Measurement of lactate is rapid, minimally invasive, and has potential to be a useful marker of perfusion in neonatal dogs. However, lactate concentrations of neonatal dogs in this study were significantly higher than those of adult dogs. Reference ranges for venous lactate concentrations in adult dogs should not be used for puppies younger than 70 days of age.

(Keywords: hypoperfusion, lactic acidosis, neonatal dogs, perfusion, pyruvate)

Introduction

Assessment of perfusion can be challenging in both human and veterinary patients, particularly neonates. In veterinary practice, mucous membrane color, capillary refill time, heart rate, pulse quality, body temperature, urinary output, and central venous pressure (CVP) are commonly used to assess perfusion and volume status. Hemodynamic parameters can vary markedly in neonates compared with adults. Pulse quality can be difficult to assess and body temperature is lower in neonatal puppies compared with adults (approximately 96–97 °F [35–36 °C]) and may not reach 100 °F (37 °C) until 2 weeks of age. CVP is higher in puppies than in adult dogs and requires placement of a central venous catheter. CVP was 8 cm H2O in normal puppies at 1 month of age and decreased to 2 cm H2O by 9 months of age in a study of 5 dogs. Concentration and dilution of urine in response to changes in the extracellular fluid volume is decreased in neonates and increases with age. In puppies at 2 days of age, the glomerular filtration rate (GFR) was only 14% of adult dog values and did not normalize until 10 weeks of age in one study. Therefore, pulse quality, body temperature, CVP, and urine output may be inadequate assessments of perfusion in neonates.

Blood lactate concentrations are considered by some to be accurate indicators of inadequate tissue perfu-
ion. Lactate concentrations have been shown to be a superior index of hypoxia when compared with oxygen delivery (DO₂), oxygen consumption (VO₂), the oxygen extraction ratio, and cardiac index (the cardiac output per minute per square meter of body surface area) in clinical studies of critically ill humans. In studies where no significant differences existed between survivors and non-survivors for DO₂ and VO₂, blood lactate concentrations were closely correlated with survival in humans and are also thought to be a more accurate prognostic indicator. Numerous studies suggest that lactate measurements are superior to measurement of CO with regards to predicting outcome in critically ill humans and that elevated lactate concentrations are linked to increased mortality rates.

Measurement of lactate concentration is a minimally invasive technique that can be accomplished using venous blood specimens.

The purpose of this study was to determine a reference range for venous lactate concentrations in neonatal dogs and to assess whether these values change as the puppies mature. To the authors’ knowledge, lactate values have not been reported for healthy neonatal puppies.

Materials and Methods

Animals

Sixty-eight clinically healthy mixed breed puppies were studied. Each of the puppies had a normal physical examination. The puppies were from 10 litters and were concurrently enrolled in a nutrition study in which the dams were fed 4 different diets. Each litter was also fed the same diet as the dam when the puppies began to eat solid foods and upon weaning at approximately 6 weeks of age. All diets were formulated to be nutritionally complete for all life stages and identical in total fat, protein, nitrogen-free extract, vitamins, and minerals. The protein content was 21% of metabolizable energy or 30% on a dry matter basis. The fatty acid composition varied among the diets. The puppies were handled daily from birth and were restrained for several minutes each day beginning at 2 weeks of age to acclimate them for the study. The research protocol was approved by the Laboratory Animal Care Committee, Texas A&M University. Venous blood samples were also taken from 30 clinically healthy adult, client-owned dogs. All adult dogs were reported to be free of disease by the owner and were normal on physical examination. The clinical research protocol was approved by the Clinical Research Review Committee, Texas A&M University. Additional data collected from each puppy included birth method (i.e. vaginal versus caesarean delivery), diet, and gender.

Samples

Blood samples were obtained from the puppies at 4, 10, 16, 28, 70, and 80 days of age. These sampling intervals were determined by the nutrition study in which the puppies were enrolled. Since litters were born at different times and several samples were clotted, not all puppies were sampled at all time points. A 23 g butterfly catheter attached to a 3 mL syringe was used for puppies at 4 and 10 days of age. A 22 g needle attached to a 3 mL syringe was used after Day 10. All venipuncture was performed by the same technician and there was minimal struggling during restraint. One dog was eliminated from the study due to excessive struggling during restraint. A single venous blood sample was taken from each of the 30 adult dogs. Blood from all dogs was collected into lithium heparin via jugular venipuncture with the animal under minimal restraint, and lactate concentration was measured immediately using an automated analyzer. Plasma lactate was assayed in whole blood via a platinum electrode that senses hydrogen peroxide. The conversion of lactate and oxygen to pyruvate and hydrogen peroxide was catalyzed by a membrane bound lactate oxidase. Electrode performance was analyzed every 2, 4, or 6 hours, depending upon the number of samples run. Internal standards were used to verify slopes and 3 standard solutions with lactate concentrations of 0.5–1.0, 2.5–3.1, and 7.0–8.4 mmol/L were analyzed daily. Precision of the machine was determined by measuring the 3 known lactate samples daily for 10 days giving an intraclass correlation coefficient of 0.9996.

Statistical analysis

Comparison of lactate concentrations in puppies of different ages with those of adult dogs was made with 6 2-sample (unequal variance) t-tests using the Bonferroni adjustment of the p-value. A p-value of 0.0083 (0.05/6) was considered significant. Tests of normality were carried out using the Shapiro–Wilks test and normal probability plots. The adult data were normally distributed and the box plots revealed symmetry without outliers. Box plots of the puppy data revealed a few outliers on Day 28 (the 95th percentile was 4.90–6.40). However, since the t-test is robust to mild deviation from normality with large sample sizes (over 25) the assumption of normality was accepted. Three-way analysis of variance (ANOVA) was used to test for the influence of birth method (i.e. vaginal versus caesarean delivery), diet, and gender on puppy lactate concentrations for each time point. Confidence intervals (CIs) are reported at the 95% level and are calculated using the t-distribution. Statistical analyses were performed using commercially available software. Data are reported as mean ± standard deviation.
Results

Blood lactate concentration was 1.80 ± 0.84 mmol/L in the 30 clinically healthy adult dogs. A total of 247 blood samples from puppies were analyzed. Blood lactate concentration was 3.83 ± 1.38 mmol/L for the puppies at 4 days of age. The mean lactate concentration for the puppies from 10 to 28 days of age was 2.70 ± 0.95 mmol/L. Lactate concentrations of the puppies were significantly higher on days 4, 10, 16, and 28 of age compared with those of healthy adults (Table 1). There was no difference between lactate concentrations of puppies at 70 or 80 days of age and those of the adult dogs.

The ANOVA showed no evidence of an effect of the 3 independent factors, birth method, diet, or gender on lactate concentrations in the puppies.

The reference range for lactate concentration for puppies at 4 days of age was 1.07–6.59 mmol/L. The 95% CI of the lower value was [0.304, 1.842] and the 95% CI of the higher value was [5.817, 7.356]. The reference range for lactate concentrations for puppies from 10 to 28 days of age was 0.80–4.60 mmol/L. The 95% CI of the lower value was [0.522, 1.082] and the 95% CI of the higher value was [4.325, 4.858].

Discussion

In these healthy puppies, venous lactate concentrations were significantly higher than those of adult dogs for the first 28 days of life. The reference range for the lactate concentration of the puppies at 4 days of age (1.07–6.59 mmol/L) was higher than the reference range for the lactate concentration of the puppies from 10 to 28 days (0.80–4.60 mmol/L). By 70 days of age, lactate concentrations were statistically indistinguishable from those of the adult dogs. Lactate concentrations in the adult dogs used in this study are similar to those reported in previous studies of healthy adult dogs.

Evans et al. reported a mean lactate concentration of 1.11 mmol/L (range 0.42–3.58 mmol/L) in 60 healthy Beagle dogs, aged 5–9 months. Hughes et al. reported mean lactate concentrations of 1.57 ± 0.47 mmol/L from the cephalic vein of 60 normal adult dogs.

Elevated lactate concentrations can reflect increased production, as occurs in hypoperfused states, or decreased elimination, as occurs in severe liver disease. Other sources of elevated blood lactate include alkalosis (due to stimulation of glycolysis), thiamine deficiency (prevents pyruvate from entering the mitochondria), and liver failure (the liver is responsible for clearance of the majority of lactate from the blood). Resolution of lactic acidosis, however, generally reflects an improvement in cell function regardless of the initial cause of the elevated lactate concentration and can, therefore, be a valuable prognostic tool to practitioners. Another cause of increased lactate production is skeletal muscle lactate which can be increased during struggling, trembling, or seizures.

Lactate concentrations have been used as indicators of decreased perfusion and as predictors of the development of multiple organ failure in humans. Lactate concentrations are also used as prognostic indicators in critically ill humans and animals. Lactate has been reported in veterinary medicine as a predictor of gastric necrosis in dogs with gastric dilatation volvulus. In human patients with septic shock, serial determinations of lactate concentration appear to be good predictors of the development of multiple organ failure and are used to guide the efficacy of treatment strategies. The duration of lactic acidosis appears to be a more accurate predictor than the initial lactate concentration in humans. Plasma lactate concentrations have been reported as predictors of death in human neonates requiring extracorporeal membrane oxygenation.

Lactate concentrations are higher in foals at birth (2.38 ± 1.03) than at 24 (1.24 ± 0.33) and 48 hours (1.08 ± 0.27) of age. In a study on baboon newborns, mean lactate was 2.33 ± 0.26 (SE) 4–6 hours after birth and decreased to 1.93 ± 0.20 at 6 weeks of age. Cerebral uptake of lactate was noted at birth but not at 6 and 12 weeks of age in this study. In a study of newborn infants, lactate was 2–3 times higher (2.9 ± 0.2 mmol/L) in 24 term newborns than it was in those beyond the neonatal period, and was even higher in the small for gestational age pre-term newborns (4.5 ± 1.2 mmol/L).

There are several possible reasons for the higher lactate concentration in neonates compared with adult dogs. In the newborn, the blood brain barrier is permeable to lactic acid, which is available to the brain in

<table>
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<th>Age (days)</th>
<th>n</th>
<th>Mean (mmol/L)</th>
<th>Std. dev</th>
<th>95% CI on difference</th>
<th>p-value</th>
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<td>1.17</td>
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<td>0.84</td>
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n, number of puppies sampled at that day of age and CI, confidence interval.
amounts exceeding that of other metabolites, including glucose.\textsuperscript{30,31} In addition, the capacity for hepatic gluconeogenesis is quite limited at birth.\textsuperscript{30} Some have speculated that lactate can act as a cerebral metabolic fuel in the neonatal period, serving as an alternative fuel during periods of hypoglycemia.\textsuperscript{25,32} Studies of newborn animals show that lactate readily enters the brain and cerebral spinal fluid in the early neonatal period.\textsuperscript{31} Hellmann et al. showed that brain/blood lactate ratios declined by 83\% during hypoglycemia in neonatal dogs, indicating that the lactate was being actively consumed by the brain during the period of hypoglycemia.\textsuperscript{32} It is possible that lactate is higher during this time period as a prophylactic protective mechanism in case the neonate undergoes hypoglycemia. Stohrer et al. suggest that the postnatal rise in lactate concentration is secondary to ischemia or reperfusion injury during birth.\textsuperscript{9} Lactate concentrations have been shown to vary according to sample site in a study of 60 healthy dogs. In this study, mean lactate concentration was highest when sampled from the cephalic vein (1.57 ± 0.47 mmol/L) followed by the femoral artery (1.43 ± 0.52 mmol/L) and the jugular vein (1.25 ± 0.49 mmol/L).\textsuperscript{18} All of the samples from the puppies in this study were taken from the jugular vein so sample site variation should not be affected.

Struggling during restraint can also increase lactate concentrations and, although struggling was minimal in these puppies, it may have contributed to the elevated lactate concentrations in these puppies.\textsuperscript{21} One human study focused on removal of lactate during periods of lactic acidosis via hemofiltration or administration of dichloroacetate (i.e. stimulates pyruvate dehydrogenase, thereby increasing oxidation of lactate), but no difference in outcome had been shown.\textsuperscript{33} The reason for the elevated lactate (i.e. decreased perfusion) seems to be the determining factor as the elevated lactate itself may not be harmful, but may just be a marker of decreased perfusion.

There are many clinical scenarios requiring fluid resuscitation to neonatal puppies (e.g. trauma, shock, etc.). Because it is especially difficult to assess perfusion indices in neonates, lactate may be particularly useful as an indicator of perfusion. Lactate trends are especially useful to help gauge therapeutic endpoints. Fluid requirements are higher in neonates due to a higher percentage of total body water, a greater surface area to body weight ratio, lack of body fat, higher metabolic rate, and a decreased ability of the kidneys to concentrate urine.\textsuperscript{34} Because the kidneys cannot concentrate urine effectively in this age group, urine output may be a questionable measure of perfusion in neonates.\textsuperscript{35,36} In addition, many of the compensatory mechanisms that are maximally stimulated in adults during shock are not fully functional in neonates.\textsuperscript{37} Mean arterial blood pressure is lower in puppies (i.e. averages 49 mmHg at 8 weeks of age) and CVP is higher at 4 weeks of age (8 cm H\textsubscript{2}O).\textsuperscript{4} Due to normal physiologic variation and small size, measurement of perfusion can be especially difficult in this age group. Lactate may, therefore, be an ideal marker of perfusion in this clinical setting. Using these reference ranges for puppies may allow clinicians to assess effects of fluid therapy on perfusion in this age group.

Limitations of this study include an absence of lactate measurements between 28 and 70 days of age. Further investigation of lactate concentrations in dogs during this time period would make it possible to model the decline of lactate levels over time. This information might help to pinpoint the age at which lactate concentrations normalize. Lactate concentrations in dogs should be investigated further, particularly during this time period. Other studies are needed to evaluate lactate concentrations in sick neonatal dogs.

In these neonatal dogs, lactate concentration was significantly higher than adult values when measured before 28 days of age. Adult reference ranges for lactate should not be used for lactate concentrations from neonatal dogs younger than 28 days of age.

\section*{Acknowledgements}

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\section*{Footnotes}

\begin{itemize}
  \item[b] Nova Stat Profile M, Nova Biomedical, Waltham, MA.
  \item[c] Stata Corporation, College Station, TX.
\end{itemize}

\section*{References}

Venous lactate concentrations