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EVALUATION OF SERIAL BLOOD LACTATE AND THE USE OF A POINT-OF-CARE LACTATE METER IN LIVE-STRANDED PINNIPEDS

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Abstract: Live-stranded pinnipeds often present to rehabilitation centers systemically debilitated with dehydration and poor perfusion. In many terrestrial mammals, blood lactate elevation has been correlated with global tissue hypoxia and decreased circulating blood volume. Serial blood lactate measurements in companion animals and humans have been used to guide fluid resuscitation therapy and evaluate prognosis. The primary objective of this study was to evaluate the prognostic value of serial blood lactate levels in live-stranded pinnipeds in a rehabilitation setting. The secondary objectives were to evaluate the use of a point-of-care lactate meter, and potassium oxalate–sodium fluoride (gray-top) tubes for storing samples for lactate analysis in pinniped patients. Fifty-five live-stranded pinnipeds (30 northern elephant seals [*Mirounga angustirostris*], 21 Pacific harbor seals [*Phoca vitulina richardsi*], and four California sea lions [*Zalophus californianus*]) that presented to a rehabilitation center were manually restrained for blood collection and lactate measurement using a point-of-care analyzer (Lactate Plus™) prior to fluid or other medical therapy. Lactate measurements were repeated 72 hr post admission and prior to euthanasia or release. Plasma samples from gray-top tubes were frozen for 30–100 days at –80°C, then thawed and evaluated using both the LactatePlus and bench-top (ABL 815) analyzers. The point-of-care analyzer was successful in measuring lactate in pinnipeds. Gray-top tubes were effective at preserving lactate levels in frozen plasma samples for up to 100 days. Released animals had significantly greater lactate clearance at 72 hr ($P = 0.039$) than animals that died or were euthanized. Therefore, lactate clearance, determined by serial blood lactate measurements, may be useful for evaluating prognosis in live-stranded pinnipeds. Initial lactate and lactate values prior to euthanasia or release were not significantly associated with outcome. Given these findings, clinicians should interpret isolated lactate values in pinnipeds with caution.

Key words: Lactate clearance, LactatePlus, *Mirounga angustirostris*, pinniped rehabilitation, *Phoca vitulina richardsi*, *Zalophus californianus*.

INTRODUCTION

Blood lactate, a product of anaerobic metabolism, is used as a marker of global tissue hypoxia and impaired tissue perfusion in critically ill patients. Initial lactate measurements have been used prognostically in humans suffering from shock of multiple etiologies.[42] Elevated lactate levels in humans have been associated with an increased risk for the development of acute respiratory distress syndrome and multiple organ dysfunction.[2,19,35,46] Serial lactate measurements and lactate clearance, as defined by ([initial lactate – serial lactate measurement] × 100 / initial lactate) is highly predictive of outcome in humans hospitalized in an Intensive Care Unit (ICU).[2,41] Specifically, failure to clear lactate is strongly associated with increased mortality in critically ill humans.[1,40]

Multiple studies have evaluated blood lactate changes in domestic species. Elevation in initial lactate in horses (*Equus ferus caballus*) with 360-degree colonic volvulus was associated with increased mortality.[30] Lactate was shown to be an early predictor of hypovolemia in horses with experimental blood loss.[36] Both initial lactate and percent change in lactate during hospitalization have been shown to be predictors of survival in dogs (*Canis familiaris*) with gastric dilatation and volvulus.[3,16,54] Dogs presenting to an emergency room with an elevated lactate 6 hr post admission were 16 times more likely to die, and failure to clear lactate by at least 50% at 6 hr post admission was strongly associated with mortality.[49]

Studies evaluating blood lactate have also been performed in a diverse range of zoological species. Blood lactate levels (using point-of-care or bench-

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top analyzers) have been measured in moose (*Alces alces*), rhinoceros (*Ceratotherium simum*), captive flamingos (*Phoenicopterus ruber*), and free-ranging avian species to determine the effects of different types of immobilization or restraint methods on circulating lactate.[7,8,22,24] Serial lactate monitoring has been performed in stranded juvenile loggerhead sea turtles (*Caretta caretta*) and three species of fish. In sea turtles, lactate values decreased over time following presentation and fluid therapy, and fish lactate levels post surgery were higher in individuals that did not survive.[9,23]

Lactate has been studied extensively in several diving species including penguins and pinnipeds to investigate normal anaerobic metabolism and physiology of the dive response.[5,6,15,44] In diving harbor seals (*Phoca vitulina*), lactate levels increased nine fold following a 10 min dive, and normalized within 40 min.[15] Seals also have a greater tissue distribution of lactate dehydrogenase isoenzymes compared with terrestrial mammals and their cardiac muscle is uniquely adapted to participate in anaerobic metabolism.[5,6] Furthermore, based on the atypical distribution of lactate dehydrogenase isoenzymes within the brain, it is suspected that seals are relatively tolerant of cerebral hypoxia during extended dives.[28]

For the present study, it was hypothesized that a pinniped's inherent ability to process and clear lactate under normal physiologic conditions could also apply to the metabolism of lactate under pathologic conditions, leading to a lower calculated lactate clearance (due to a lower initial lactate value) compared with terrestrial mammals. Normal blood lactate levels for wild pinnipeds have been reported.[43,50] One study evaluated blood lactate in eight immobilized, recently weaned, healthy northern elephant seal pups (*Mirounga angustirostris*) before and after the postweaning fast; mean lactate levels were 3.21 mM/L and 1.98 mM/L, respectively.[50] A second study measured lactate in 30 healthy, juvenile Galápagos sea lions (*Zalophus wollebaeki*) in order to develop normal reference intervals. The mean reported blood lactate in this study was 2.7 mM/L.[43] Despite the abundance of data describing lactate levels during the dive response and normal physiologic conditions, there have been no studies that have evaluated lactate levels in clinically ill pinnipeds.

Hundreds to thousands of seals and sea lions live-strand along the California coast every year. According to the National Oceanic and Atmospheric Association (NOAA), 3,340 California sea lions (*Zalophus californianus*) stranded off the coast of California between the months of January and May in 2015 alone.[39] Many of these live-stranded pinnipeds are presented to marine mammal rescue and rehabilitation centers. These facilities are tasked with the management of critically ill animals, many of which require aggressive medical interventions. Common causes of live-stranding in pinnipeds in California include malnutrition, parasitic infections, and trauma[2,21]—all conditions that can negatively impact tissue perfusion and lead to hyperlactatemia. In these cases, resuscitation therapy is often directed at restoring circulating blood volume through the use of crystalloid fluids. As blood volume increases, tissue perfusion is restored, and lactate is cleared from the blood stream.[40]

The primary objective of the study reported here was to evaluate the relationship between lactate clearance, using serial blood lactate values (prior to, and after resuscitation therapy), and survival in live-stranded pinnipeds in a rehabilitation setting. The secondary objectives were to evaluate the use of a portable, poolside, lactate meter and potassium oxalate–sodium fluoride (gray-top) tubes as a means of measuring and preserving pinniped lactate levels, respectively.

MATERIALS AND METHODS

Point-of-care serial lactate evaluations

The study protocol was approved by the Institutional Animal Care and Use Committees at The University of California, Davis (Protocol No. 19677) and The Marine Mammal Center (TMMC), Sausalito, California. Pacific harbor seals (HS; *Phoca vitulina richardsi*), northern elephant seals (NES; *Mirounga angustirostris*), and California sea lions (CSL; *Zalophus californianus*) that stranded within a 600-mile range of Northern and Central California coastline and presented to TMMC were eligible for the study. On presentation to the rehabilitation clinic, all animals were manually restrained for a physical examination, mentation assessment (bright, quiet, obtunded, stuporous, or comatose), and blood collection. The age, species, and sex of every individual admitted into the study were recorded. Based on examination findings, a general primary cause of stranding for each animal was designated using the following categories: malnutrition, maternal separation, trauma, infectious disease, toxicity, and other.

A venous blood sample was collected prior to administration of any medications or fluids (intravenous, subcutaneous, oral) and prior to feeding. For blood collection, study animals were restrained for less than 5 min to collect the sample, and no more than three attempts to acquire the sample were performed. Blood was collected either with the use of a needle (18–22 ga, 1–1.5 inch depending on patient size) and syringe (3–12 ml) or by using a vacutainer system (Becton Dickinson, Franklin Lakes, NJ 07417, USA) into a single 6-ml potassium oxalate–sodium fluoride (gray-top) tube (Becton Dickinson). Sampling sites included the extradural vein (NES and HS) and the caudal gluteal vein (CSL). Harbor seal and California sea lion samples were run within 5 min by placing one drop of whole blood from the gray-top tube directly onto the portable LactatePlus™ analyzer (Nova Biomedical, Waltham, MA 02454, USA) test strip. If any of the blood was clotted in the tube before lactate was measured, the samples were discarded without analysis. Due to the presence of high hematocrit values (normal for this species but higher than is typical for companion animals), northern elephant seal whole blood samples would routinely result in error messages on the LactatePlus meter. Therefore, all NES blood samples were centrifuged for 10 min at 3,000 × g and lactate was measured using plasma. Following point-of-care lactate measurement, blood samples from HS and CSL were also centrifuged at 3,000 × g for 10 min, and all plasma was collected and stored at –80°C for further analysis.

Following the initial blood sample, all animals received parenteral fluids for at least 3 days, in addition to enteral tube feedings according to previously established TMMC protocols. For HS (weight range 5–12 kg), fluid therapy included either Lactated Ringer's Solution (LRS) with 2.5% dextrose (Baxter, Deerfield, IL 60015, USA), or 0.45% NaCl with 2.5% dextrose (Baxter) subcutaneously (SC), at 250 ml twice daily. Northern elephant seals that were 30–35 kg received 500 ml 0.9% sodium chloride (Baxter) SC twice daily; animals that were 35–40 kg received 750 ml SC twice daily. California sea lions up to 15 kg received LRS (Baxter) 250 ml SC twice daily; animals between 15 and 20 kg received 500 ml SC twice daily. For every additional 5 kg body weight, CSLs were administered an additional 250 ml SC.

Venous blood samples for serial point-of-care lactate measurement were repeated as above for study animals at approximately 72 hr post admission to the center as well as within 24 hr of euthanasia or being cleared for release.

LactatePlus and potassium oxalate–sodium fluoride tube evaluation

Forty-three frozen plasma samples, representing a range of values (0.3–12.8 mM/L), from all three species, were thawed for the lactate evaluation study. Within 5 min of reaching room temperature, the plasma lactate was determined for each sample by using both the LactatePlus analyzer and an established bench-top lactate analyzer[18] (Radiometer ABL 815 Blood Gas Analyzer, 2700 Bronshoj, Denmark) at the University of California, Davis Veterinary Medical Teaching Hospital (VMTH). The prefreezing and postthaw LactatePlus values were compared to determine the effectiveness of gray-top tubes for preserving pinniped lactate samples. The postthaw LactatePlus values were compared with the bench-top analyzer to determine the effectiveness of the LactatePlus meter for pinniped lactate evaluation. The percent bias for each evaluation study was then determined using the difference of the means from paired samples.

Statistical analysis

Statistical analysis was performed using Stata IC/13.1 software (StataCorp LLC, College Station, TX 77845, USA). Changes in initial and serial lactate measurements for each species of pinniped were evaluated using the Kruskal–Wallis nonparametric analysis of variance, with comparisons based on two traits: (1) their final disposition—i.e., whether they were ultimately released or were euthanized or died at the facility, and (2) one of six primary causes of stranding. P-values $<$ 0.05 were considered statistically significant. For the meter and tube evaluation studies, linear regression analysis was performed to calculate a Pearson correlation coefficient. Passing and Bablok regression was performed to further compare the methods using MedValc software (MedCalc, v. 17.6, 9030 Mariakerke, Belgium). Bland–Altman plots were also generated to evaluate potential bias, which plotted the differences in the prefreezing and postthaw LactatePlus values, as well as the differences in postthaw LactatePlus and postthaw bench-top ABL 815 analyzer versus the means of the respective two values.

RESULTS

Serial lactate evaluation

From April to June 2016, initial lactate measurements were performed in 55 live-stranded pinnipeds (30 NES, 4 CSL, 21 HS) that met inclusion criteria for admittance into the study. Sex distribution, age class, outcome, and cause of stranding for all study animals are depicted in Table 1. Causes of stranding listed as "other" included ocular disease ($n = 3$), congenital abnormalities ($n = 2$), premature birth ($n = 1$), and gastrointestinal perforation ($n = 1$). "Infectious" causes included *Otostrongylus* sp. parasitic pneumonia ($n = 5$), *Parafilaroides* sp. parasitic pneumonia ($n = 1$), and fungal gastritis ($n = 1$). The single "neoplasia" case was an animal with urogenital carcinoma. Forty-four study animals had a second lactate measurement performed at 72 hr post presentation and 34 lactate measurements were performed in study animals prior to euthanasia or release. Mean lactate values at each time point and for each species are shown in Table 2. Lactate values for both outcomes at all time points are depicted in Table 3. Both absolute and percent lactate clearance are shown in Table 4. Released animals had significantly greater lactate clearance at 72 hr ($P = 0.039$) than animals that died or were euthanized. All other parameters evaluated, including presenting lactate, lactate prior to euthanasia or release, sex, age class, mentation, and cause of stranding were not significantly associated with outcome.

LactatePlus and potassium oxalate–sodium fluoride tube evaluation

Plasma samples from 43 potassium oxalate–sodium fluoride tubes, frozen for 30 to 100 days, were thawed and evaluated for the meter and tube evaluation studies. Twenty-two HS and 21 NES plasma samples, with a wide range of lactate values (0.3–12.8 mM/L), were submitted for analysis. Prefreezing LactatePlus values were positively correlated with postthaw LactatePlus values. Linear regression of the prefreezing and postthaw LactatePlus values showed a correlation coefficient of 0.93. Passing and Bablok linear regression analysis had a slope of 1.33 (95% confidence interval [CI]: 1.12–1.55), an intercept of –0.53 (95% CI: –0.84 to –0.11), and an $R^2 = 0.92$. The Bland–Altman plot of the potassium oxalate–sodium fluoride tube evaluation study (Fig. 1) showed a mild proportional bias. Calculated percent bias for the tube evaluation study was 11.14%. Postthaw LactatePlus values were positively correlated with postthaw bench-top (ABL 815) lactate values. Linear regression of the postthaw LactatePlus and bench-top ABL 815 lactate values showed a correlation coefficient of 0.97. Passing and Bablok linear regression analysis had a slope of 1.09 (95% CI: 1.00–1.21), an intercept of –0.20 (95% CI: –0.37 to 0), and an $R^2 = 0.96$. The Bland–Altman plot of the LactatePlus evaluation study (Fig. 2) showed a mild proportional bias. Calculated percent bias for the meter evaluation study was 0.49%.

DISCUSSION

This study showed that a greater 72-hr blood lactate clearance was associated with a positive outcome (release) in live-stranded pinnipeds at a rehabilitation center. Animals that were released had an average negative trend in their blood lactate (–0.16 mMol/L), compared with an average positive lactate trend (+0.35 mM/L) in pinnipeds that died or were euthanized. Based on these findings, patients that fail to show a decrease in blood lactate following 72 hr of appropriate resuscitation therapy (parenteral fluids and enteral nutrition), may have a worse prognosis for release. These results are consistent with similar studies in humans and companion animals. In humans with severe sepsis and septic shock, 24-hr lactate clearance was the best predictor of 28-day mortality rate.[38] Further, a greater lactate clearance within the first 6 hr of admittance to an ICU was associated with decreased mortality in septic people and those with significant trauma.[42,51]

Lactate clearance serves as a surrogate for both the magnitude and duration of global tissue hypoxia and is associated with an overall improvement in inflammatory biomarkers and organ dysfunction scoring.[40] Blood lactate elevation results from decreased oxygen delivery capabilities in the body during circulatory shock. Oxidative phosphorylation declines as mitochondrial hypoxia occurs, and anaerobic glycolysis becomes the primary means for energy metabolism. Lactate is produced within the cell as a result of anaerobic gylcolysis, which ultimately enters the blood stream as cells adapt to a hypoxic environment.[32,52] Lactate clearance is a reflection of increased tissue oxygenation, often secondary to an increase in circulating blood volume, following the administration of crystalloid fluids. Fluid therapy can restore adequate peripheral perfusion, allowing cells to convert back to oxidative phosphorylation, thereby decreasing anaerobic glycolysis and the production of lactate.[32]

Along with being used diagnostically and prognostically, lactate clearance is used in humans as a guide for early goal-directed therapy for patients in septic shock.[1,40] It has been as useful as central venous saturation, another established

Table 1. Sex, age class, cause of stranding, and outcome for northern elephant seals (*Mirounga angustirostris;* NES), California sea lions (*Zalophus californianus;* CSL), and Pacific harbor seals (*Phoca vitulina richardsi;* HS) that presented live-stranded to a rehabilitation facility and met inclusion criteria for the study. Age class was determined using a combination of standard body length, tooth development, and life history stages.

	NES	CSL	HS	Total
Sex				
Male	18	2	11	31
Female	12	2	10	24
Age class				
Pup	0	2	21	22
Weanling	30	0	0	30
Juvenile	0	0	0	0
Subadult	0	1	0	1
Adult	0	1	0	1
Cause of stranding				
Malnutrition	17	0	0	17
Maternal separation	0	0	18	18
Infectious disease	8	1	1	10
Toxin	0	0	0	0
Trauma	0	1	1	2
Neoplasia	0	1	0	1
Other	5	1	1	7
Outcome				
Release	21	1	16	38
Death or euthanasia	9	3	5	17

resuscitation end point, as a parameter for goal-directed therapy.[31] The generally accepted target clearance in humans is >10% lactate improvement at a minimum of 2 hr, with lactate normalization being the most powerful indicator of resuscitation success.[31] One study placed the optimal survival cut-off value as high as 36% lactate improvement.[51] In systemically ill dogs, a lactate decrease of >50% six hr after the onset of resuscitation was associated with increased survival.[49] In the present study, animals that survived showed a mean lactate decrease of 2.6%, while patients that died or were euthanized had a mean increase in their blood lactate of 48.4%. These data suggest that a significant yet reduced lactate clearance, when compared with terrestrial mammals, may be expected in ill pinnipeds. Further, the degree of lactate increase should be considered alongside lactate improvement when evaluating prognosis in live-stranded pinnipeds.

In this study, the ability to clear lactate, albeit by a lower percentage than has shown to be prognostic in people and dogs, was associated with a positive outcome. This finding is not surprising, as live-stranded pinnipeds often present to rehabilitation centers dehydrated and with evidence of poor perfusion. There is a general paucity of published prognostic indicators for stranded marine mammals under medical care. A recent study showed that cardiac troponin 1 levels were increased in harbor seal (*Phoca vitulina*) pups that died, while in pups that survived, troponin 1 levels decreased over the course of rehabilitation.[20] Similarly, the present study aims to equip wildlife veterinarians with an additional prognostic parameter for pinnipeds undergoing medical care at rehabilitation facilities.

When interpreting the pinniped lactate clearance in this study, the temporal and technical differences from human and companion animal studies should be acknowledged. Previous studies have evaluated lactate clearance from 6 hr up until 24 hr post admission, with fluids being administered by the intravenous route. In the present study, fluids were primarily administered SC for logistical and practical reasons. A 72-hr postadmission time point was elected for the second lactate measurement to allow for multiple SC fluid treatments to be administered prior to the second lactate evaluation. It is therefore unknown if any significant changes to the blood lactate occurred following the first administration of fluids up until 72 hr later. However, from a

Table 2. Mean ± standard deviation serial lactate of northern elephant seals (*Mirounga angustirostris*), California sea lions (*Zalophus californianus*), and Pacific harbor seals (*Phoca vitulina richardsi*) that presented live-stranded to a rehabilitation facility. Measurements were performed at presentation, 72 hr post admission and prior to euthanasia or release. The number of animals at each time point is included in parentheses.

	Serial lactate measurement (mM/l)		
Species	Initial	72 hr	Euthanasia or release
NES	1.28 ± 0.46 (30)	1.36 ± 0.53 (24)	2.86 ± 1.3 (20)
CSL	2.1 ± 0.91 (4)	1.45 ± 0.21 (2)	0.9 (1)
HS	1.64 ± 0.61 (21)	1.46 ± 0.80 (18)	2.96 ± 1.8 (13)
All	1.48 ± 0.60 (55)	1.41 ± 0.64 (44)	2.84 ± 1.5 (34)

Table 3. Mean ± standard deviation serial lactate measurements of northern elephant seals (*Mirounga angustirostris*), California sea lions (*Zalophus californianus*), and Pacific harbor seals (*Phoca vitulina richardsi*) that presented live-stranded to a rehabilitation facility. Measurements were performed at presentation, 72 hr post admission, and prior to euthanasia or release. The number of animals at each time point is included in parentheses. Serial lactate measurements are striated based on outcome.

	Serial lactate measurement by outcome (mM/L)					
Species	Initial, alive	Initial, deceased	72 hr, alive	72 hr, deceased	Release	Death or euthanasia
NES	1.33 ± 0.46 (21)	1.49 ± 0.47 (9)	1.24 ± 0.33 (17)	1.67 +/1 0.79 (7)	2.7 ± 1.3 (17)	3.7 ± 1.7 (3)
CSL	2.3 (1)	2.03 ± 1.1 (3)	1.3 (1)	1.6 (1)	0.9 (1)	
HS	1.73 ± 0.63 (16)	1.32 ± 0.41 (5)	1.5 ± 0.87 (15)	1.3 ± 0.36 (3)	2.96 ± 1.8(13)	
All	1.58 ± 0.58 (38)	1.36 ±0.65 (17)	1.36 ± 0.63 (33)	1.56 ±0.65 (11)	2.75 ± 1.5(31)	3.7 ± 1.7 (3)

subjective clinical perspective, pinnipeds at the 72-hr time point were better hydrated and perfused than at admission, making 72-hr lactate clearance a reasonable parameter to evaluate.

While lactate clearance was significantly associated with outcome, initial lactate and lactate values prior to euthanasia or release were not. This is in contrast to studies in humans and companion animals that have shown prognostic value (although typically less than serial lactate measurements) for initial lactate values, prior to resuscitation therapy. A potential reason for this is the inherent ability of pinnipeds to tolerate hypoxia and anaerobic glycolysis as a result of their unique physiologic adaptations that make up the dive response. Adaptations for prolonged dives in seals and sea lions include bradycardia, peripheral vasoconstriction, apnea, and changes in the distribution of cardiac output.[26] These adaptations result in peripheral tissue hypoperfusion, anaerobic glycolysis, and lactate generation under normal physiologic conditions. Following just 7 min under water, tissue oxygen and glycogen stores—the primary substrates for aerobic glycolysis—are depleted and lactate production increases.[15] Historical simulated diving experiments have shown that the peak lactate production does not occur during the dive due to a relative systemic hypometabolism, but rather shortly after the dive during the initial recovery period, once peripheral tissues are re-perfused.[15] When compared with mice, hooded seals (*Cystophora cristata*) also had a three-fold increase in cerebral glycogen stores, providing additional fuel for aerobic metabolism and delaying the onset of anaerobic generation of blood lactate.[14] Once lactate is produced, an enhanced distribution (compared with terrestrial mammals) of lactate dehydrogenase isoenzymes helps to promote the clearance and normalization of blood lactate. The summation of these adaptations may contribute to a pinniped's ability to process and clear lactate even while dehydrated and malperfused and may explain the lack of association between initial lactate and outcome in this study.

Initial lactate levels were lower overall in dehydrated live-stranded pinnipeds compared with ill companion animals. A mean initial lactate for seals and sea lions that died in the present study was 1.49 mM/L, while a study in dogs that died after presentation to an ICU had a mean initial lactate of 5.07 mM/L.[35] This further supports the hypothesis that pinnipeds are able to process and clear lactate under pathologic conditions better than terrestrial mammals, and in connection with the serial lactate data in this

Table 4. Mean ± standard deviation serial lactate clearance, striated by outcome, of northern elephant seals (*Mirounga angustirostris*), California sea lions (*Zalophus californianus*), and Pacific harbor seals (*Phoca vitulina richardsi*) that presented live-stranded to a rehabilitation facility. Released animals had significantly greater lactate clearance at 72 hr ($P = 0.039$) than animals that died or were euthanized. Percent lactate clearance was determined using ([initial lactate − serial lactate measurement] × 100 / initial lactate). The number of animals at each time point is included in parentheses.

Species	Lactate clearance alive (mM/L)	Lactate clearance deceased (mM/L)	Lactate clearance alive (%)	Lactate clearance deceased (%)
NES	−0.05 ± 0.43 (17)	0.6 ± 0.91 (7)	+5.89 ± 48.8 (17)	+71.07 ± 100.6 (7)
CSL	−1 (1)	0.6 (1)	−43.5 (1)	+60 (1)
HS	−0.24 ± 0.84 (16)	−0.23 ± 0.68 (5)	−9.43 ± 51.7 (16)	−8.44 ± 40.6 (5)
All	−0.16 ± 0.66 (38)	0.37 ± 0.86 (17)	−2.3 ± 49.7 (38)	+48.4 ± 88.0 (17)

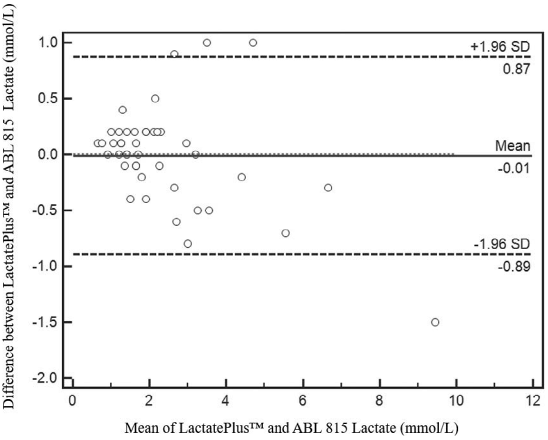

Figure 1. Bland–Altman plot for lactate values of pinnipeds obtained with the LactatePlus meter immediately after sample collection (before freezing) and after thawing following frozen storage at −80°C for 30–100 days (post thaw). Bias (solid line) and limits of agreement (dashed lines) and are shown. Other than one outlier, the values were within or nearly within the limits of agreement.

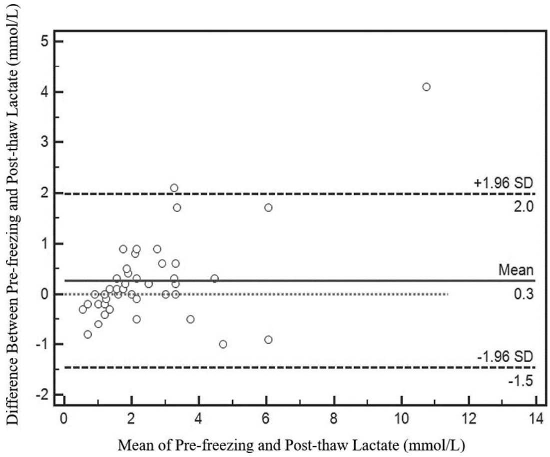

Figure 2. Bland–Altman plot for pinniped serum lactate values obtained using the LactatePlus and ABL 815 bench-top analyzers following frozen storage of samples at −80°C for 30–100 days (post thaw). Bias (solid line) and limits of agreement (dashed lines) are shown. Other than one outlier, the values were within or nearly within the limits of agreement.

study, exemplifies the importance of using lactate clearance to assess prognosis, rather than evaluating single lactate measurements in isolation.

Interestingly, lactate levels in surviving animals prior to release were higher (mean 2.75 mM/L) than their initial lactate (mean 1.58 mM/L). In order to be candidates for release, these animals had finished a course of treatment and were considered systemically healthy by veterinary staff. Lactate levels in manually restrained cats (*Felis catus*) have been shown to temporarily spike secondary to agitation, excitement, and prolonged restraint.[11,45,47] Every attempt to limit the time and force required to restrain animals for blood collection was used in this study. However, when compared with the debilitated condition of most study subjects at presentation, animals had gained significant size and strength through the course of their rehabilitation, requiring more firm restraint for safe blood collection prior to release. This restraint likely resulted in a sudden spike in blood lactate levels that the animals were not able to clear by the previously described mechanisms prior to blood collection. The impact of restraint may explain the lack of significance between outcome and lactate prior to euthanasia or release. While caution in the interpretation of a single lactate measurement is again emphasized, the release lactate for these 31 stranded pinnipeds may be considered as a general guideline for normal healthy pinniped lactate levels in a rehabilitation setting.

The present study demonstrated that the point-of-care LactatePlus analyzer was effective in measuring lactate in pinnipeds. This portable meter has previously been validated in humans[25,34] and has been used in Galápagos sea lions (*Zalophus wollebaeki*), pinfish (*Lagodon rhomboids*), and American martens (*Martes Americana*).[13,43,48] A point-of-care lactate meter has applications for both wild and captive pinnipeds, providing near instantaneous results without the need for bulky laboratory equipment.

Potassium oxalate–sodium fluoride tubes were effective at preserving lactate levels in frozen pinniped plasma samples for up to 100 days. These tubes are considered the gold standard for blood lactate preservation in people.[17] In humans, lactate concentrations remain stable for up to 8 hr at room temperature and over 24 mo once frozen.[4,18] These tubes include potassium oxalate as an anticoagulant and sodium fluoride as a preservative agent. While not evaluated in this study, potassium oxalate–sodium fluoride tubes have been used in human medicine as a means to preserve glucose levels as well.[10] These tubes have been used in various species, including for glucose preservation in Northern fur seals (*Callorhinus ursinus*), sea otters (*Enhydra lutris*), and other wildlife.[27,29,37,53] This study shows the further potential field utility of potassium oxalate–sodium fluoride tubes for use in wildlife blood collection and delayed lactate analysis.

Limitations to this study include sample size and age class constraints. Pups are generally the most common HS age class, and weanlings the most common NES age class presenting live-stranded to The Marine Mammal Center. California sea lions present with a more diverse age distribution, however only a limited number of this species presented for rehabilitation during the study period. There is little published information on the effect of age on lactate production under pathologic conditions; however, in exercising horses lactate did not significantly change with age.[33] Inclusion criteria for the study included blood sampling prior to fluid therapy, which is generally more technically challenging, especially in debilitated and dehydrated animals, from the caudal gluteal vein (as is performed in sea lions) compared with the extradural vein used in HS and NES, thus decreasing the number of animals available for inclusion in this study. Additionally, larger sea lions (greater than approximately 60 kg) usually require sedation or full anesthesia for examination rather than manual restraint, which could have an additional effect on lactate values, thus these animals were not included in the study. Limitations of the meter evaluation study included lack of quality control data (quality control was performed but not recorded) from the LactatePlus and ABL815 analyzers during the course of this study. While percent bias was computed, the lack of quality control data prevented evaluation and calculation of total error. However, the veterinary guideline for allowable total error is 40% and given the low percent bias in this study (0.49%), it is likely that the bias plus twice precision would fall under this limit. Further studies would be needed to verify this suggestion.

Future study considerations would include further evaluating lactate clearance in pinnipeds and other diving species. Investigations comparing deep diving (NES) and more shallow diving (CSL) marine mammals may elucidate the degree to which diving adaptations influence lactate clearance. All animals in this study were administered parenteral fluids by species-specific protocols. Future studies may look into the effect that different resuscitation protocols may have on lactate clearance and outcome.

CONCLUSION

The present study shows the effectiveness of the LactatePlus meter for lactate measurement in pinnipeds and potassium oxalate–sodium fluoride tubes for preserving lactate levels in frozen plasma samples for up to 100 days. The results from this study suggest that clinicians should interpret isolated lactate values in pinnipeds with caution, and that there is likely greater clinical utility in evaluating serial blood lactate values in these species. Lactate clearance may be considered when assessing overall prognosis in live-stranded pinnipeds undergoing rehabilitation.

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LITERATURE CITED

1. Arnold RC, Shapiro NI, Jones AE, Schorr C, Pope J, Casner E, Parillo JE, Dellinger RP, Trzeciak S. Multicenter study of early lactate clearance as a determinant of survival in patients with presumed sepsis. Shock. 2009;32(1):35–39.

2. Bakker J, Gris P, Coffernils M, Kahn RJ, Vincent JL. Serial blood lactate concentrations can predict the development of multiple organ failure following septic shock. Am J Surg. 1996;171:221–226.

3. Beer KA, Syring RS, Drobatz KJ. Evaluation of plasma lactate concentration and base excess at the time of hospital admission as predictors of gastric necrosis and outcome and correlation between those variables in dogs with gastric dilatation-volvulus: 78 cases (2004–2009). J Am Vet Med Assoc. 2013;242(1): 54–58.

4. Blanco F, Garcia-Benayas T, Jose de la Cruz J, Gonzalez-Lahoz J, Soriano V. First-line therapy and mitochondrial damage: different nucleosides, different findings. HIV Clin Trials. 2003;4:11–19.

5. Blix AS, Berg T, Fyhn HJ. Lactate dehydrogenase in a diving mammal, the common seal (*Phoca vitulina vitulina*). Int J Biochem. 1969;1(3):292–294.

6. Blix AS, From SH. Lactate dehydrogenase in diving animals—a comparative study with special reference to the eider (*Somateria mollissima*). Comp Biochem Physiol. 1971;40B: 579–584.

7. Burgdorf-Moisuk A, Wack R, Zicardi M, Larsen RS, Hopper K. Validation of lactate measurement in American flamingo (*Phoenicopterus ruber*) plasma and correlation with duration and difficulty of capture. J Zoo Wildl Med. 2012;43(3):450–458.

8. Buss P, Olea-Polpeka F, Meyer L. Evaluation of cardiorespiratory, blood gas, and lactate values during

extended immobilization of white rhinoceros (*Ceratotherium simum*). J Zoo Wildl Med. 2015;46(2):224–233.

9. Camacho M, Quintana MP, Calabuig P, Luzardo O, Boada LD, Zumbado M, Oros J. Acid-base and plasma biochemical changes using crystalloid fluids in stranded juvenile loggerhead sea turtles (*Caretta caretta*). PLoS One [Internet]. 2015 [cited 2015 November 15]; Available from doi:10.1371/journal.pone.0132217

10. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem. 1989;35(2):315–317.

11. Christopher MM, O'Neill S. Effect of specimen collection and storage on blood glucose and lactate concentrations in healthy, hyperthyroid and diabetic cats. Vet Clin Pathol. 2000;29:22–28.

12. Colegrove KM, Greig DJ, Gulland FMD. Causes of live strandings of northern elephant seals (*Mirounga angustirostris*) and Pacific harbor seals (*Phoca vitulina*) Along the Central California coast, 1992–2001. Aquat Mamm. 2005;31(1):1–10.

13. Collins S, Dornburg A, Flores JM, Dombrowski DS, Lewbart GA. A comparison of blood gases, biochemistry, and hematology to ecomorphology in a health assessment of pinfish (*Lagodon rhomboids*). PeerJ [Internet]. 2016 [cited 2015 November 11];4: e2262. Available from doi:10.7717/peerj.2262

14. Czech-Dumal NU, Geiseler M, Hoff MLM, Schliep R, Ramirez JM, Folkow LP, Burmester T. The role of glycogen, glucose and lactate in neuronal activity during hypoxia in the hooded seal (*Cystophora cristata*) brain. Neuroscience. 2014;275:374–383.

15. Davis, RW. Lactate and glucose metabolism in the resting and diving harbor seal (*Phoca vitulina*). J Comp Physiol. 1983;V153:275–288.

16. de Papp E, Drobatz KJ, Hughes D. Plasma lactate concentration as a predictor of gastric necrosis and survival among dogs with gastric dilatation-volvulus: 102 cases (1995–1998). J Am Vet Med Assoc. 1999;215(1):49–52.

17. Dube MP, Kitch DW, Parker RA, Alston-Smith BL, Mulligan K. The effect of long-term storage on measured plasma lactate concentrations and prospective lactate results from a multicenter trial of antiretroviral therapy. Clin Chem Lab Med. 2005;43(9):947–952.

18. Ebner PAR, Romano P, Saint'Anna A, Mendes ME, Oliveira M, Sumita NM. Validation protocol for multiple blood gas analyzers in accordance with laboratory accreditation programs. J Bras Patol Med Lab. 2015;51(5):296–302.

19. Fenwick JC, Dodek PM, Ronco JJ, Phang PT, Wiggs B, Russell JA. Increased concentrations of plasma lactate predict pathologic dependence of oxygen consumption on oxygen delivery in patients with adult respiratory distress syndrome. J Crit Care. 1990; 5:81–86.

20. Fonfara S, Sundermeyer J, Casamian SD, Weber C, Rosenberger T. Usefulness of serum cardiac troponin I concentration as a marker of survival of harbor seal (*Phoca vitulina*) pups during rehabilitation. J Am Vet Med Assoc. 2016;249(12):1428–1435.

21. Greig DJ, Gulland MD, Kreuder C. A decade of live California sea lion (*Zalophus californianus*) strandings along the Central California coast. Aquat Mamm. 2005;31(1):11–22.

22. Haga HA, Wenger S, Hvarnes S. Plasma lactate concentrations in free-ranging moose (*Alces alces*) immobilized with etorphine. Vet Anaesth Analg. 2009;36(6):555–561.

23. Hanley CS, Clyde VL, Wallace RS, Paul-Murphy J, Patterson TA, Keuler NS, Sladky KK. Effects of anesthesia on serial blood gas values and lactate concentrations in yellow perch (*Perca flavescens*), walleye pike (*Sander vitreus*), and koi (*Cyprinus carpio*). J Am Vet Assoc. 2010;236(10):1104–1108.

24. Harms CA, and Harms RV. Venous blood gas and lactate values of mourning doves (*Zenaida macroua*), boat-tailed grackles (*Quiscalus major*), and house sparrows (*Passer domesticus*) after capture by mist net, banding, and venipuncture. J Zoo Wildl Med. 2012; 43(1):77–84.

25. Hart S, Drevets K, Alford M, Salacinski A, Hunt BE. A method-comparison study regarding the validity and reliability of the LactatePlus analyzer. BMJ Open [Internet]. 2013 [cited 2015 November 21];3:e001899. Available from doi:10.1136/bmjopen-2012-001899

26. Hochachka PW. Pinniped diving response mechanism and evolution: a window on the paradigm of comparative biochemistry and physiology. Comp Biochem Physiol Part A. 2000;126:435–458.

27. Hoff GL, McEldowney LE, Bigler J, Kuhns J, Tomas JA. Blood and urinary values in the gray squirrel. J Wildl Dis. 1976;12:349–352.

28. Hoff MLM, Fabrizius A, Folkow LP, Burmester T. An atypical distribution of lactate dehydrogenase isoenzymes in the hooded seal (*Cystophora cristata*) brain may reflect a biochemical adaptation to diving. J Comp Physiol B. 2016;186:373–386.

29. Hunter L, Madin SH. Clinical blood values of the northern fur seal, *Callorhinus ursinus.* II. Comparison of fresh versus stored frozen serum. J Wildl Dis. 1978;14:116–119.

30. Johnston K, Holcombe SJ, Hauptman JG. Plasma lactate as a predictor of colonic viability and survival after 360 degrees volvulus of the ascending colon in horses. Vet Surg. 2007;36(6):563–567.

31. Jones AE. Lactate clearance for assessing response to resuscitation in severe sepsis. Acad Emerg Med. 2013;20(8):844–847.

32. Jones AE, Shapiro NI, Trzeciak S, Arnold RC, Claremont HA, Kline JA. Lactate clearance vs central venous oxygen saturation as goals of early sepsis therapy: a randomized clinical trial. J Am Med. Assoc. 2010;303(8):739–746.

33. Kang OD, Park YS. Effect of age on heart rate, blood lactate concentration, packed cell volume and hemoglobin to exercise in Jeju crossbreed horses. J Anim Sci and Technol. 2017;59:2.

34. Kulandaivlan S, Verma SK, Mukhopadhyay S, Vignesh N. Test retest reproducibility of a hand-held lactate analyzer in healthy men. J Exerc Sci Physiol. 2009;5(1):30–33.

35. Lagutchik MS, Ogilvie GK, Hackett TB, Wingfield WE. Increased lactate concentrations in ill and injured dogs. J Vet Emerg Crit Care. 1996;8(2):117–127.

36. Magdesian KG, Fielding CL, Rhodes DM, Ruby RE. Changes in central venous pressure and blood lactate concentration in response to acute blood loss in horse. J Am Vet Assoc. 2006;229(9):1458–1462.

37. Mainka SA. Hematology and serum biochemistry of captive swift foxes (*Vulpes velox)*. J Wildl Dis. 1988;24(1):71–74.

38. Marty P, Roquilly A, Vallee F, Aymeric L, Ferre F, Fourcade O, Asehnoune K, Minville V. Lactate clearance for death prediction in severe sepsis or septic shock during the first 24 hours in intensive care: an observational study. Ann Intensive Care. 2013;3(1):3.

39. National Oceanic and Atmospheric Organization [Internet]. 2015 Elevated California sea lions strandings in California; c2015 [cited 2018 March 1]. Available from http://www.westcoast.fisheries.noaa.gov/mediacenter/faq_2015_ca_sea_lion_strandings.pdf

40. Nguyen HB, Loomba M, Yang JJ, Jacobsen G, Shah K, Otero RM, Suarez A, Parekh H, Jaehne A, Rivers EP. Early lactate clearance is associated with biomarkers of inflammation, coagulation, apoptosis, organ dysfunction and mortality in severe sepsis and septic shock. J Inflamm (Lond). 2010;7:6.

41. Nguyen HB, Rivers EP, Knoblich BP, Jacobsen G, Muzzin A, Ressler JA, Tomlanovich MC. Early lactate clearance is associated with improved outcome in severe sepsis and septic shock. Crit Care Med. 2004; 32(8):1637–1642.

42. Odom SR, Howell MD, Silva GS, Nielsen VM, Gupta A, Shapiro NI, Talmor D. Lactate clearance as a predictor of mortality in trauma patients. J Trauma Acute Care Surg. 2013;74(4):999–1004.

43. Paez-Rosas D, Hirschfeld M, Deresienski D, Lewbart GA. Health status of Galápagos sea lions (*Zalophus wollebaeki*) on San Cristóbal Island rookeries determined by hematology, biochemistry, blood gases, and physical examination. J Wildl Dis. 2016;52(1):100–105.

44. Ponganis PJ, Kooyman GL, Winter LM, Starke LN. Post-dive blood lactate concentrations in emperor penguins (*Aptenodytes forsteri*). J Exp Biol. 1997;200: 1623–1626.

45. Rand JS, Kinnaird E, Baglioni A, Blackshaw J, Priest J. Acute stress hyperglycemia in cats is associated with struggling and increased concentrations of lactate and norepinephrine. J Vet Intern Med. 2002;16: 123–132.

46. Roumen RM, Redl H, Schlag G, Santdner W, Koller W, Goris RJ. Scoring systems and blood lactate concentrations in relation to the development of adult respiratory distress syndrome and multiple organ failure in severely traumatized patients. J Trauma. 1993;35:349–355.

47. Siragusi RHS, Barros VTM, Fiorini EA, Bergamo TM, Siqueira RC, Manhoso FFR, Franco RP. Measurement of serum lactate values in domestic cats (*Felis catus*) submitted to physical and chemical restraint. Braz J Vet Res Anim Sci. 2017;54(4):383–387.

48. Spriggs MC, Muller LI, Keenlance P, Sanders RL, Witt JC, Miller DL. Effects of live-trapping and isoflurane anesthesia on free-ranging American martens (*Martes Americana*). J Wildl Dis. 2017;53(3):447–458.

49. Stevenson CK, Kidney BA, Duke T, Snead ECR, Mainer-Jamie RC, Jackson ML. Serial blood lactate concentrations in systemically ill dogs. Vet Clin Pathol. 2007;36:234–239.

50. Tavoni SK, Champagne CD, Houser DS. Lactate flux and gluconeogenesis in fasting, weaned northern elephant seals (*Mirounga angustirostris*). J Comp Physiol B. 2013;183:537–546.

51. Walker CA, Griffith DM, Gray AJ, Datta D, Hay AW. Early lactate clearance in septic patients with elevated lactate levels admitted from the emergency department to intensive care: time to aim higher? J Crit Care. 2013;28:832–837.

52. Watts JA, Kline JA. Bench to bedside: the role of mitochondrial medicine in the pathogenesis and treatment of cellular injury. Acad Emerg Med. 2003;10(9): 985–997.

53. Williams TD, Pulley LT. Hematology and blood chemistry in the sea otter (*Enhydra lutris*). J Wildl Dis. 1983;19(1):44–47.

54. Zacher LA, Berg J, Shaw SP, Kudej RK. Association between outcome and changes in plasma lactate concentration during presurgical treatment in dogs with gastric dilatation-volvulus: 64 cases (2002–2008). 2010;236(8):892–897.

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