The Effect of Oxygen Tension, Syringe Type and Temperature on Arterial Blood Gas Storage in Healthy Alpacas

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Abstract: *Objective*: To assess the influence of storage temperature, syringe type and oxygen tension on arterial blood gas (ABG) parameters in alpacas.

Study Design: Prospective, randomized, cross-over study.

Animals: Six healthy, adult alpacas (2-8 years).

Methods: All alpacas were randomly exposed to supplemental oxygen, room air, and hypoxic oxygen/nitrogen gas mixtures via a sealed facemask and non-rebreathing circuit, following recovery from sedation (0.1 mg kg-1 IV xylazine) for saphenous arterial catheterization. For each gas type, 16 ABG samples were collected into heparinized glass and plastic syringes, with eight stored at room temperature and eight in iced water (3-4°C). Samples were analyzed at 5, 30, 60 and 120 minutes after collection and evaluated via repeated measures ANOVA and paired samples t-test ($P<0.05$).

Results: The PaO_2 significantly decreased at room temperature in both normoxemic and hyperoxemic blood, following storage for ≥30 minutes in glass syringes, and in hyperoxemic blood stored in plastic syringes at room temperature. However, the mean PaO_2 of hyperoxemic blood stored in plastic syringes on ice significantly increased at all time-points. Overall, two hour storage induced a mean change of <3.4 mmHg, <1.2 mEq L-1 and <0.03 in $PaCO_2$, HCO_3- and pH, respectively. Cold storage in glass syringes resulted in the smallest mean alteration in PaO_2 (≤10.28 mmHg).

Conclusions and Clinical Relevance: ABG from alpacas should be stored on ice in glass syringes to reduce variability in PaO_2 if analysis is delayed. In contrast to human reports, the PaO_2 of cooled hyperoxygenated camelid blood increases following storage in plastic syringes.

Keywords: Arterial blood gas, storage, alpacas, llamas, oxygenation.

INTRODUCTION

The accurate assessment of respiratory dysfunction requires an objective characterization of oxygenation and ventilation, as the clinical manifestation of respiratory disease may be subtle in llamas and alpacas. Arterial blood gas (ABG) analysis has thus gained increasing importance in the diagnosis and management of respiratory illness in these camelids. However, most affected patients reside on farms without access to on-site arterial blood gas analysis, thus requiring the transport and storage of arterial blood.

Depending on species, there is conflicting evidence as to the clinical utility and validity of stored arterial blood samples. The American Association for Respiratory Care recommends against the use of blood gas samples stored in plastic syringes for longer than 30 minutes [1]. Canine arterial blood, however, has been shown to have a stable PaO_2 and $PaCO_2$ for up to 6 hours in plastic syringes stored on ice [2]. In contrast, a study of equine arterial blood concluded that the PaO_2 should be analyzed immediately, or evaluated within 2 hours after sampling if obtained in glass syringes and subsequently stored on ice. In the latter study, the PaO_2 was rapidly affected by both syringe type and storage temperature; conversely $PaCO_2$ and pH measurements remained accurate for up to 1 hour in samples stored at room temperature regardless of the syringe type used [3]. A more recent study assessing equine ABG documented very similar findings to the former publication [4]. In contrast, PaO_2 in bovine arterial blood has been shown to undergo no clinically relevant changes when stored on ice in plastic syringes for up to 24 hours prior to analysis [5]. Previous research has shown that the stability of ABG samples may also vary depending on the arterial blood oxygen tension. In humans, samples with a high partial pressure of oxygen show a rapid decrease in PaO_2 over time [6].

Bearing in mind these conflicting results, it is important to obtain species-specific recommendations on the storage and interpretation of arterial blood gases. Historically, South American Camelids are high-

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altitude adapted animals, whose red blood cells have a greater affinity for oxygen and a high oxygen transfer conductance [7]. Furthermore, the oxygen-hemoglobin dissociation curve is shifted to the left in these camelids and their blood oxygen content is reportedly higher when compared to low altitude species such as sheep [8]. These findings may specifically impact changes in PaO_2 in stored ABG of alpacas. In addition, a wide range of PaO_2 values are encountered in different disease states, which may further alter the stability of PaO_2 in stored blood gas samples, as previously shown in humans [6]. The goal of the current study was, therefore, to evaluate the effects of both oxygen tension and storage condition on ABG in the evolutionally high-altitude adapted alpaca. These data may serve as a baseline to determine the utility of arterial blood gas storage in camelids where immediate sample analysis is not possible.

MATERIALS AND METHODS

The study protocol was approved by the Clinical Sciences Research Committee of the Cummings School of Veterinary Medicine at Tufts University. Written owner consent was obtained for all animals prior to enrollment.

Six adult (age: 2-8 years), client-owned alpacas (4 male, 2 female) were assessed as healthy based on physical examination, hematology and clinical history. Xylazine was administered intravenously (0.1 mg kg^{-1}, Lloyd Laboratories, Shenandoah, IA) to facilitate placement of an arterial catheter. With the animal in temporary lateral recumbency, an area over the saphenous artery was clipped and aseptically prepared. An 18-gauge over the needle catheter (BD Insyte™ Autoguard™, BD Medical, Sandy, Utah, USA; 1.3 x 48 mm) was subsequently placed percutaneously into the artery using standard technique, and secured in place. An extension set (Extension Set, Baxter Healthcare Corporation, Deerfield, IL, USA; inner volume: 2.3 ml) was attached to the catheter and flushed with heparinized saline to facilitate sampling once the animal was awake.

Following recovery to standing, all alpacas were randomly exposed to room air (N), hyperoxygenated breathable gas (H; oxygen/nitrogen ratio of 4:3) and hypoxic gas mixtures (L; nitrogen/oxygen ratio of 15:3) for a period of 10 minutes to ensure equilibration prior to ABG sampling. More specifically, the order of test gas exposure was determined *via* arbitrary match of each alpaca with one of 6 possible test gas arrangements (NHL, NLH, HNL, HLN, LHN, LNH). The gas mixtures were provided through a tight fitting, sealed facemask attached *via* a nonrebreathing apparatus to a 10L reservoir bag. The gases were transferred to the reservoir bag using standard regulators. A washout period of 15 minutes was allowed between the administration of each gas mixture.

For each gas mixture, 16 arterial blood samples were randomly collected following withdrawal of a 10 mL blood aliquot, which was discarded. Eight samples were collected into glass syringes (5 mL Fortuna Optima, Poulten & Graf GmbH, Wertheim, Germany), and eight into plastic syringes (Terumo®, Terumo Corporation, Somerset, NJ) which had previously been heparinized (Heparin Sodium Injection, 1.000 USP units mL^{-1}, APP Pharmaceuticals, Schaumburg, IL). All syringes were immediately capped with an aluminum lined airtight plastic stopper (Kendall Monoject Tip Caps, Tyco Healthcare Group LP, Mansfield, MA). Half of all samples per group were stored at room temperature (22 ^{o}C) and half were stored in iced water (3-4^{o}C) for a period of up to 2 hours. One sample from each group (plastic syringe stored at room temperature, plastic syringe on ice, glass syringe at room temperature and glass syringe on ice) was analyzed for pH, bicarbonate, lactate, PaO_2 and $PaCO_2$ using a point of care blood gas analyzer (Stat Profile Xpress Blood Gas Analyzer, NOVA Biomedical, Waltham, MA, USA) at 5 (baseline – time 0), 30, 60 and 120 minutes following sample collection. The expected accuracy of the Stat Profile Xpress Blood Gas Analyzer was 99.4 – 99.7% based on the manufacturer's data. Priming, calibration, and quality control were ensured *via* SmartCheck Automated Maintenance. Samples were mixed by gentle rolling immediately prior to analysis. Following completion of the protocol, all arterial catheters were removed and the site bandaged for a period of 1 hour.

A univariate repeated measures analysis of variance (ANOVA) was used at baseline to determine syringe type and storage temperature effects, and syringe and temperature interaction effects. Additionally, all data were analyzed using a univariate two groups repeated measures ANOVA to assess syringe type, storage temperature and time effects, and syringe x time and temperature x time interaction effects. Since a significant syringe x time and temperature x time interaction was observed for the evaluated arterial blood gas variable, each ABG value was compared with baseline values separately.

Changes in pH, PaO_2, $PaCO_2$, HCO_3- and lactate between baseline and stored samples (30, 60 and 120 minutes) were therefore reported descriptively as Δ change (mean differences +/- standard deviation), and compared between time-points using a paired samples T-test. Normality of data distribution was verified using the Kolmogorov-Smirnov analysis. All analyses were performed using standard software (SPSS 15.0, SPSS Inc., Chicago, Ill). Values of $P < 0.05$ were considered significant.

RESULTS

The mean arterial blood gas (ABG) parameters obtained from healthy adult alpacas exposed to

Table 1: Mean ± SD ABG Parameters of Healthy, Adult Alpacas (n=6) Exposed to Normoxic, Hypoxic and Hyperoxic Inspired Gas Mixtures

Blood gas tension	pH	PaO_2	$PaCO_2$	HCO_3^- (mEq L^{-1})	Lactate (mmol L^{-1})
Hypoxemic	7.42 ± 0.05	52.2 ± 8.9 mmHg 6.94 ± 1.18 kPa	38.4 ± 12.3 mmHg 5.11 ± 1.64 kPa	24.5 ± 4.8	0.75 ± 0.20
Normoxemic	7.47 ± 0.02	106.2 ± 9.5 mmHg 14.12 ± 1.26 kPa	28.2 ± 3.6 mmHg 3.75 ± 0.48 kPa	20.7 ± 2.8	0.55 ± 0.09
Hyperoxemic	7.44 ± 0.04	227.3 ± 40.5 mmHg 30.23 ± 5.39 kPa	31.8 ± 5.2 mmHg 4.23 ± 0.69 kPa	21.8 ± 2.5	0.41 ± 0.24

Table 2: Mean ± SD Changes in ABG Parameters of *hypoxemic* Adult Alpacas (n=6), Based on Storage Type (Plastic vs. Glass), Storage Temperature and Time

	0 – 30 minutes	0 – 60 minutes	0 – 2 hours
pH			
Plastic – RT	-0.01 ± 0.01	-0.01 ± 0.01	-0.01 ± 0.01
Plastic – iced	-0.00 ± 0.01	-0.00 ± 0.01	-0.00 ± 0.01
Glass – RT	-0.01 ± 0.01	-0.01 ± 0.02	-0.01 ± 0.02
Glass – iced	-0.00 ± 0.01	-0.00 ± 0.01	-0.00 ± 0.01
PaO_2			
Plastic – RT	0.7 ± 6.3	1.7 ± 9.1	4.0 ± 10.8
Plastic – iced	0.8 ± 7.2	4.1 ± 12.5	4.7 ± 11.7
Glass – RT	-1.1 ± 7.9	2.1 ± 9.4	0.6 ± 9.5
Glass – iced	0.7 ± 8.8	0.6 ± 9.3	3.1 ± 11.8
$PaCO_2$			
Plastic – RT	1.2 ± 2.3	1.9 ± 1.7	-0.2 ± 2.3
Plastic – iced	1.4 ± 2.5	1.2 ± 2.0	-0.1 ± 2.3
Glass – RT	0.8 ± 2.6	0.7 ± 2.4	-0.3 ± 2.8
Glass – iced	0.8 ± 2.9	0.0 ± 1.0	-0.6 ± 1.8
HCO_3^-			
Plastic – RT	0.5 ± 1.2	0.6 ± 0.8	-0.8 ± 1.0
Plastic – iced	0.8 ± 1.3	0.7 ± 0.8	-0.1 ± 1.3
Glass – RT	0.3 ± 1.3	0.1 ± 0.9	-0.8 ± 1.2
Glass – iced	0.7 ± 1.4	-0.1 ± 1.0	-0.2 ± 1.1
Lactate			
Plastic – RT	0.08 ± 0.08	0.14 ± 0.11[a]	0.28 ± 0.19[a]
Plastic – iced	-0.05 ± 0.11	-0.03 ± 0.12	-0.05 ± 0.10
Glass – RT	0.06 ± 0.04	0.14 ± 0.11[a]	0.24 ± 0.16[a]
Glass – iced	-0.07 ± 0.08	-0.05 ± 0.11	-0.09 ± 0.12

[a]Denotes a significant difference from baseline (P<0.05); RT – room temperature; (-) – indicates a reduction in blood gas values from baseline.

Table 3: Mean ± SD Changes in ABG Parameters of *normoxemic* Adult Alpacas (n=6), Based on Storage Type (Plastic vs. Glass), Storage Temperature and Time

	0 – 30 minutes	0 – 60 minutes	0 – 2 hours
pH			
Plastic – RT	-0.02 ± 0.01[a]	-0.02 ± 0.01[a]	-0.03 ± 0.01[a]
Plastic – iced	-0.01 ± 0.02	-0.01 ± 0.02	-0.01 ± 0.00[a]
Glass – RT	-0.02 ± 0.01[a]	-0.03 ± 0.01[a]	-0.03 ± 0.02[a]
Glass – iced	-0.00 ± 0.01	-0.01 ± 0.02	-0.01 ± 0.01[a]
PaO_2			
Plastic – RT	-1.4 ± 9.0	-7.1 ± 12.5	-2.2 ± 12.0
Plastic – iced	5.8 ± 13.3	8.0 ± 12.5	15.8 ± 20.3
Glass – RT	-12.6 ± 10.3[a]	-18.1 ± 7.8[a]	-17.2 ± 10.1[a]
Glass – iced	-2.8 ± 6.5	-6.1 ± 13.2	0.1 ± 9.7
$PaCO_2$			
Plastic – RT	1.3 ± 1.4	2.2 ± 2.3	3.2 ± 2.7
Plastic – iced	1.9 ± 2.6	1.8 ± 2.6	2.0 ± 3.0
Glass – RT	1.6 ± 2.1	2.4 ± 1.7[a]	3.4 ± 2.3[a]
Glass – iced	0.1 ± 2.4	1.6 ± 2.2	2.5 ± 3.3
HCO_3^-			
Plastic – RT	0.2 ± 0.6	0.4 ± 1.0	0.7 ± 1.2
Plastic – iced	1.1 ± 1.6	0.7 ± 1.2	0.9 ± 2.2
Glass – RT	0.5 ± 1.1	0.5 ± 0.9	0.8 ± 1.0
Glass – iced	-0.1 ± 1.6	0.7 ± 1.0	1.2 ± 2.4
Lactate			
Plastic – RT	0.08 ± 0.05	0.15 ± 0.06[a]	0.27 ± 0.12[a]
Plastic – iced	0.03 ± 0.05	-0.03 ± 0.05	-0.17 ± 0.21
Glass – RT	0.10 ± 0.00[a]	0.15 ± -0.6[a]	0.30 ± 0.10[a]
Glass – iced	-0.03 ± 0.05	-0.05 ± 0.06	-0.28 ± 0.28

[a]Denotes a significant difference from baseline (P<0.05); RT – room temperature; (-) – indicates a reduction in blood gas values from baseline.

normoxic, hypoxic and hyperoxygenated inspired gas mixtures are listed in Table **1**. The effect of storage type (plastic vs. glass), temperature (iced at 4°C vs. 22°C room temperature) and blood gas tension (normoxemia, hypoxemia, hyperoxemia) are summarized in Tables **2-4**.

Significant increases in lactate concentration were only identified following blood storage at room temperature, irrespective of syringe type. Although a statistically significant increase in lactate was noted at room temperature in hypoxemic, normoxemic and hyperoxyemic blood samples over time, the mean change in blood lactate remained <0.5 mmol L^{-1} following storage for up to two hours. Similarly, statistically significant alterations in pH, $PaCO_2$ and bicarbonate were only associated with room temperature storage. Mean changes in arterial blood gas parameters remained below 3.4 mmHg for $PaCO_2$, 1.2 mEq L^{-1} for bicarbonate and a 0.03 decrease in pH after two hour storage.

The PaO_2 significantly decreased in both normoxemic and hyperoxemic blood samples, following storage in glass syringes at room temperature, irrespective of storage time point. Similar decreases in PaO_2 were observed in hyperoxygenated blood stored at room temperature in plastic syringes. In contrast, PaO_2 significantly increased after storage of hyperoxemic blood samples in plastic syringes on ice. The greatest mean alterations in PaO_2 were observed during storage at room temperature, using blood with a higher oxygen tension. Storage in glass syringes on ice resulted in the lowest variability of PaO_2, with the highest mean PaO_2 increase being 10.28 mmHg under these conditions.

Table 4: Mean ± SD Changes in ABG Parameters of Oxygen Supplemented (*hyperoxemic*) Adult Alpacas (n=6), Based on Storage Type (Plastic vs. Glass), Storage Temperature and Time

	0 – 30 minutes	0 – 60 minutes	0 – 2 hours
pH			
Plastic – RT	-0.02 ± 0.04	-0.01 ± 0.03	-0.02 ± 0.04
Plastic – iced	-0.02 ± 0.05	-0.01 ± 0.04	0.00 ± 0.04
Glass – RT	-0.02 ± 0.04	-0.02 ± 0.04	-0.02 ± 0.04
Glass – iced	-0.02 ± 0.04	-0.01 ± 0.04	0.00 ± 0.04
PaO_2			
Plastic – RT	-14.3 ± 13.2[a]	-28.2 ± 16.3[a]	-43.6 ± 27.6[a]
Plastic – iced	13.0 ± 8.1[a]	16.7 ± 11.8[a]	15.8 ± 13.0[a]
Glass – RT	-10.9 ± 7.9[a]	-19.0 ± 15.6[a]	-43.4 ± 19.5[a]
Glass – iced	7.8 ± 11.2	10.3 ± 11.0	6.6 ± 9.7
$PaCO_2$			
Plastic – RT	2.5 ± 4.3	2.2 ± 3.7	3.4 ± 3.5
Plastic – iced	2.6 ± 4.8	0.9 ± 2.7	-0.1 ± 2.4
Glass – RT	0.1 ± 5.0	1.3 ± 2.5	1.4 ± 2.4
Glass – iced	2.5 ± 4.8	0.6 ± 2.8	1.0 ± 2.4
HCO_3^-			
Plastic – RT	0.5 ± 1.3	0.5 ± 1.5	1.0 ± 1.1
Plastic – iced	0.7 ± 1.4	0.1 ± 0.9	-0.1 ± 1.0
Glass – RT	0.2 ± 1.3	-0.1 ± 0.9	-0.1 ± 1.0
Glass – iced	0.5 ± 1.6	-0.2 ± 1.0	0.5 ± 0.6
Lactate			
Plastic – RT	0.16 ± 0.16	0.18 ± 0.18	0.48 ± 0.23[a]
Plastic – iced	0.10 ± 0.12	-0.00 ± 0.04	-0.00 ± 0.30
Glass – RT	0.14 ± 0.16	0.10 ± 0.27	0.38 ± 0.21[a]
Glass – iced	0.10 ± 0.13	-0.06 ± 0.11	0.02 ± 0.20

[a]Denotes a significant difference from baseline (P<0.05); RT – room temperature; (-) – indicates a reduction in blood gas values from baseline.

DISCUSSION

The PaO_2 of arterial blood gases in alpacas should be analyzed immediately for accurate assessment of oxygenation, as PaO_2 undergoes the most extensive and rapid variations during storage. As previously described [4, 6, 9, 10], this effect was limited by storage of iced samples in glass syringes, due to the lower gas permeability of glass in comparison to plastic and the reduction of cell metabolism at low temperatures [11]. If analysis is delayed, samples should therefore be stored on ice in glass syringes, to reduce variability in PaO_2.

The stability of ABG samples may vary depending on the arterial blood oxygen tension. Normoxemic blood samples stored at room temperature in glass syringes showed a significant reduction in PaO_2 over time. This effect was likely caused by persistent aerobic metabolism of blood cells at room temperature outpacing the concentration-dependent diffusion of oxygen from room air (PO_2 of 150 mmHg at sea level) into stored samples (mean PaO_2 = 106.2 mmHg). Similar observations were made for hyperoxygenated samples stored at room temperature, irrespective of syringe type. Hyperoxemic, iced samples from alpacas, however, showed an increase in PaO_2 during storage in plastic syringes. These results stand in strict contrast to observations using human blood, where samples with a high partial pressure of oxygen showed a rapid decrease in PaO_2 over time [6]. The latter observations were attributed to oxygen metabolism by red blood cells, respiratory burst and the equilibration with atmospheric PaO_2, leading to a concentration-dependent outward diffusion of oxygen. The mean PaO_2 of hyperoxygenated alpaca blood, however,

increased from 227.3 ± 40.5 mmHg (time 0) to 243 ± 34.5 mmHg (time 120) after storage in iced plastic syringes. These species-related differences in the dissociation of oxygen may be based on genetically-coded alterations in oxygen affinity between hemoglobin of high and low altitude-adapted animals. The hemoglobin molecule of members of the South American Camelid family (e.g. llamas and alpacas) shows a high oxygen affinity, which has been attributed to substitutions of amino acid residues in the β-chain of llama hemoglobin [12]. The amino acid substitution deletes two of the seven diphosphoglycerate contacts in the tetrameric hemoglobin, which increases blood O_2 affinity by reducing the 2,3 diphosphoglycerate (DPG) effect in camelids [13]. It appears plausible that higher levels of DPG under cold storage conditions may release hemoglobin-bound oxygen in camelids. It has been shown that 2,3-DPG concentrations in red cell concentrates are higher after storage of whole blood at 4°C than 22°C, presumably related to the increased rate of glycolysis at higher temperatures [14]. As the sample temperature decreases, the Bunsen solubility coefficient of oxygen in plasma increases [15], which may further encourage dissociation of hemoglobin-bound oxygen within erythrocytes and entry into plasma. However, to the best of the authors' knowledge, the specific effect of temperature on oxygen binding has not been investigated in alpacas to date.

Storage of arterial blood for up to 2 hours leads to clinically acceptable alterations in pH, $PaCO_2$, bicarbonate ion concentration and lactate over time, irrespective of storage temperature, syringe type or blood oxygen pressure in adult alpacas. The magnitude of these storage-related changes is unlikely to affect patient management. In contrast, clinically relevant alternations and high variability in PaO_2 were observed during blood gas storage over time. Although statistically significant changes in PaO_2 were only identified in normoxemic and hyperoxemic blood, a large standard deviation in mean values may have contributed to an underestimation of statistically significant differences in PaO_2 between stored samples and baseline values. Delayed ABG analysis (within 2 hours) may, therefore, be considered an acceptable alternative for the assessment of acid-base disturbances but not oxygenation, if immediate sample processing is unavailable.

CONCLUSION

For clinical purposes, storage of arterial blood gases from alpacas for up to 2 hours on either ice or at room temperature may be acceptable for determination of pH, lactate, bicarbonate ion concentration and $PaCO_2$. Although small, statistically significant changes in these variables were observed with storage, these are unlikely to detract from the interpretation of ABG in the clinical setting. For determination of PaO_2, immediate analysis without storage is recommended. If storage cannot be avoided then sampling into a glass syringe, kept on ice is recommended to reduce variability. However, these results should be interpreted with caution, as the PaO_2 of cooled camelid blood tends to increase during storage.

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