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Arterial pH and Blood Lactate Levels of Anesthetized Mongolian Khulan (*Equus hemionus hemionus*) in the Mongolian Gobi Correlate with Induction Time

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ABSTRACT: Research and conservation of wide-ranging wild equids in most cases necessitate capture and handling of individuals. For free-roaming Mongolian khulan (*Equus hemionus hemionus*), also known as the khulan, capture involves a strenuous, high-speed chase, and physiologic responses have yet to be elucidated. We analyzed sequential arterial blood gas (ABG) samples as a proxy for respiratory and metabolic status of khulan during capture-related anesthesia. We recorded precise chase and induction times and monitored vital parameters and ABG from free-ranging khulan during anesthesia performed for GPS collaring. At the initiation of anesthesia, animals had ABG values similar to those recorded for thoroughbred horses (*Equus caballus*) after maximal exercise. Longer induction times resulted in higher arterial pH ($P<0.001$) and lower blood lactate ($P<0.002$). This trend of improvement continued over the course of anesthesia. The most important factor explaining pH and lactate was the time that elapsed between cessation of the chase and obtaining the first ABG sample, which, under field conditions, is tightly linked to induction time. All animals recovered uneventfully. Our data show that khulan recover and shift their metabolic status back toward expected normal values during opioid-based field anesthesia.

Key words: Anesthesia, arterial, equid, *Equus hemionus*, lactate, pH, wild.

The Gobi regions of southern Mongolia and northern China constitute the most important remaining stronghold of the Mongolian khulan (*Equus hemionus hemionus*). Capture and GPS collaring of khulan are pivotal in the effort to raise awareness for the large spatial requirements of khulan in the Mongolian Gobi Desert (Kaczensky et al. 2006) and the threat of habitat fragmentation by large-scale infrastructure development (Batsaikhan et al. 2014). Consequently, monitoring khulan movements to identify key habitats, and to develop and monitor mitigation measures, has become a priority in Mongolia.

In the absence of suitable helicopters and pilots for wildlife capture in this remote area of central Asia, the most efficient method of capture has been chasing and darting khulan from a pursuing vehicle at speeds of up to 60 km/h (Walzer et al. 2006). Requiring animals to exercise maximally just prior to inducing anesthesia must be regarded as a physiologic challenge (Hubbell et al. 2000). We hypothesized that longer chase times would negatively impact metabolic status during anesthesia in khulan. Even in the clinical setting, hypoxemia and hypercapnia are common events during equine anesthesia as a result of ventilation-perfusion mismatch and shunting (Wagner 2008). This problem is aggravated in remote field settings, where, in addition to the use of respiratory depressant drugs such as etorphine, supplementation of oxygen or mechanical ventilation of the patient is frequently impracticable. We sequentially sampled arterial blood to measure lactate and blood gases to investigate the physiologic consequences of capture and subsequent field anesthesia in khulan.

We collected data 24–30 August 2013 during capture and GPS collaring of 20 individuals (13 males, seven females) in two locations of the southern Gobi Desert of Mongolia (43°N, 107°E; 43°N, 109°E). Mean ambient temperatures ranged from 15.3 C to 27.7 C. Groups of or individual khulan were approached slowly and as covertly as possible in a four-wheel-drive vehicle. Once the animal started to flee, the chase was initiated, and a stopwatch was started. The time between the start of the chase and the time the dart struck the animal

TABLE 1. Descriptive means±SD and ranges (minimum–maximum) of temperature-corrected, arterial partial pressure of oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), saturation of arterial hemoglobin (SaO_2), and vital parameters measured at time point 1 and 15 min thereafter, for Mongolian khulan (*Equus hemionus hemionus*) in the Mongolian Gobi Desert.

	PaO_2 (mm Hg)		SaO_2 (%)		$PaCO_2$ (mm Hg)		Pulse rate		Respiratory rate		Body temperature	
Time	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
1	70±18	(40–102)	80±12	(54–93)	36±8	(25–49)	69±11	(52–95)	24±7	(16–40)	40.1±0.7	(38.9–41.3)
15	69±10	(50–82)	84±6	(69–90)	37±6	(25–47)	69±15	(52–103)	23±6	(12–34)	39.9±0.6	(38.4–40.9)

and the vehicle slowed down was defined as time of chase (TOC). The time between the dart hitting the animal and the animal going into anesthesia-induced recumbency was defined as induction time. The animals were immobilized with a previously described (Walzer 2014) mixture of 2.45 mg of etorphine and 10 mg of acepromazine (L.A. Immobilon, Novartis Animal Health UK Limited, Frimley, UK) in combination with 10 mg of butorphanol (Butomidor, Richter Pharma AG, Wels, Austria) and 10 mg of detomidine (Domosedan, Orion Corporation, Espoo, Finland) mixed in a 3-mL dart equipped with a 42-mm coned needle and applied with a CO_2-powered rifle (DAN-INJECT, ApS, Børkop, Denmark).

Within 90 s after induction, a pulse oximeter (Rad-57, Masimo Corp., Irvine, California, USA) was applied on the tongue, and, if possible, the first arterial blood sample was drawn anaerobically from the facial artery, designated sample point T=1. No oxygen was supplied. Subsequently, a venous blood sample was drawn, a hair sample was obtained, predefined morphologic measures (including body weight estimate) were taken, and the GPS collar was fitted. During this period, vital parameters (pulse rate, respiratory rate, arterial hemoglobin saturation as measured by pulse oximeter, and rectal body temperature [BT], measured via handheld thermometer [Veterinärthermometer SC12, K-Jump Health Co., Ltd., Taiwan]) were monitored and recorded intermittently. A second arterial blood sample from the facial artery was obtained 15 min (T=15) after T=1. Both samples were analyzed immediately with a handheld blood gas analyzer (CG4+ cartridge, i-STAT, Abbott Point of Care Inc., Princeton, New Jersey, USA). After completion of the procedure, anesthesia was reversed with 12 mg of diprenorphine (L.A. Revivon, Novartis) intravenously and 50 mg of naltrexone (Trexonil, Wildlife Pharmaceuticals Inc., Windsor, Colorado, USA) intramuscularly. All statistical tests were carried out using R 3.0.2 (R Development Core Team 2013).

Once aware of the approach, all animals fled at a full gallop. Darts were placed into the left thigh. Mean TOC was 249 (±97) s. Induction time ranged from 100 to 1,409 s (median = 338 s). In 15 of the 20 animals captured, it was possible to record all parameters and arterial blood samples

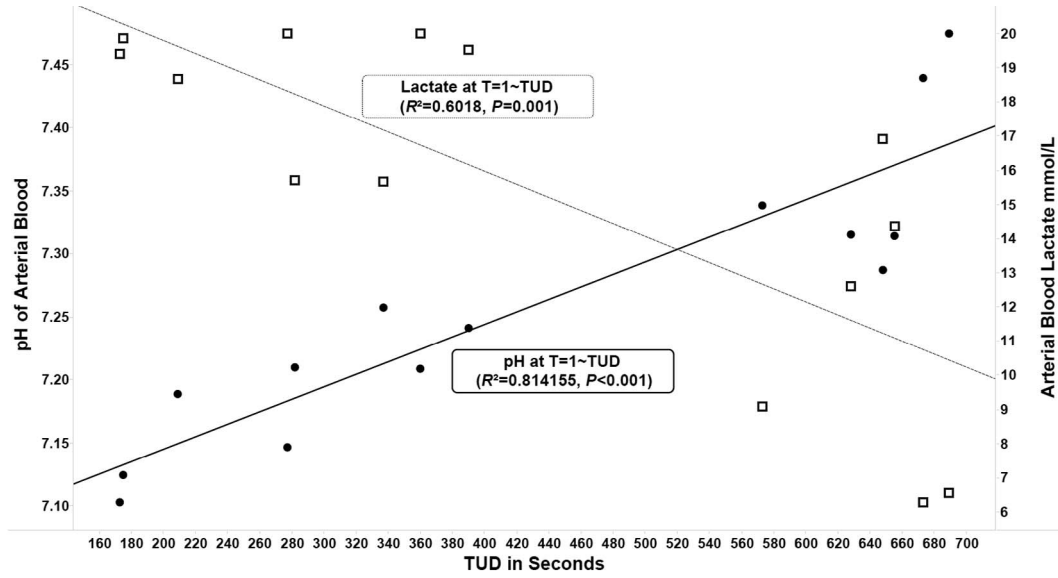

FIGURE 1. Correlation (~) of arterial lactate concentration (mmol/L), dashed line and boxes, and arterial pH, solid line and circles, at T=1 with induction time (TUD) in free-ranging Mongolian khulan (*Equus hemionus hemionus*) after a high-speed chase followed by anesthesia via remote dart with a combination of etorphine, acepromazine, butorphanol, and detomidine. Results of the linear model are presented in the respective box.

at T=1. The sequential sample at T=15 was possible in 14 animals. Vital parameters and blood gas analysis results for T=1 and T=15 are shown in Table 1. There was no effect of TOC on any observed parameters except temperature (R^2=0.2622, P=0.029, as calculated by linear model). Arterial pH was directly proportional to induction time (R^2=0.81, P<0.001, linear model), with short induction times resulting in extreme metabolic acidemia at T=1. Factors affecting blood pH were also influenced by induction time. Lactate measured at T=1 was inversely proportional to induction time; the longer an animal kept moving after darting and without being chased any longer, the lower was the measured lactate (R^2=0.6018, P<0.002, linear model; Fig. 1). This trend continued throughout immobilization (Fig. 2), with lactate decreasing and pH increasing from T=1 to T=15 (Fig. 2). Estimated weight of anesthetized animals was 210–300 kg. All animals recovered uneventfully and walked away within 240 s (median=90 s, range=23–240 s) of reversal.

Blood gas analysis is a rapid and precise method of gaining insight into the fundamental metabolic and respiratory status of an animal. Our data suggest that, once anesthetized, metabolic and respiratory status of animals was unaffected by TOC. The observed positive correlation of TOC with BT is well known, which is why chase times are generally limited (5–7 min, maximum) to avoid potentially negative consequences of severe hyperthermia (Walzer et al. 2006). The BT at T=1 was likely also affected by induction time and ambient temperatures, which can help explain the relatively weak correlation. Both metabolic and respiratory parameters measured in arterial blood at T=1 and T=15 (Table 1) showed marked divergence and, besides partial pressure of CO_2 ($PaCO_2$), were far from physiologic values for equids. The observed arterial blood gas status was also considerably worse than has been reported previously for trained horses after an exercise above maximal O_2 consumption capacity (VO_{2max}) and then anesthetization (Hubbell et al. 2000). However, our observed blood gas status was similar to that of awake thoroughbred horses after maximal exercise (Manohar et al. 2001). We hypothesize that, with exception of partial pressure of O_2 (PaO_2) and saturation of arterial hemoglobin

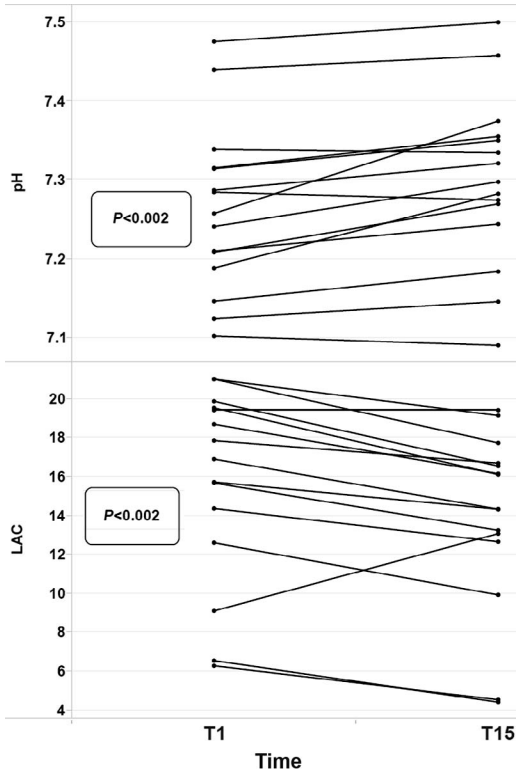

FIGURE 2. Ladder plot showing the individual trends of arterial pH and lactate (LAC) concentration (mmol/L), of free-ranging Mongolian khulan anesthetized via remote dart with a combination of etorphine, acepromazine, butorphanol, and detomidine after a high-speed chase, measured immediately after induction (T1) and 15 min thereafter (T15). Each line connects the first and second measurement in an individual. The *P* value of the respective linear mixed effect model is shown in the box.

(SaO_2; as changes in these values could theoretically have happened during the first 90 s of recumbency when no monitoring was possible), the values at T=1 are largely explained by the chase and only marginally by anesthetic effects. Consequently, blood gas values have to be evaluated within the context of the capture method, and the anesthetist needs to have a solid background in exercise physiology to draw the correct conclusions.

Our data show that transient, but extreme, metabolic derailments occur during capture events preceded by a full-speed chase. Furthermore, we show that the time that elapses until an animal becomes recumbent after it was darted (plus the time until the arterial blood sample is drawn) should be considered as the most influential factor when interpreting such samples. It is well known that lactate is rapidly released when initiating physical activity, even below VO_{2max}, and arterial concentrations subsequently decrease once exercise has ceased (Birks et al. 1991). In khulan, this trend does not seem to be altered by application of anesthetic drugs, which has been reported previously in trained domestic horses (Hubbell et al. 2000).

Depending on definition (Wagner 2008), hypoxemia was evident in 27% (PaO_2<60 mmHg) or 83% (SaO_2<90%) of measurements, and thus supplementation with oxygen remains an important goal. The wide range of $PaCO_2$, which in our study was not correlated with pH, is presumably an effect of the intense exercise. Both hypercapnia as a direct consequence of exercise and hyperventilation-induced hypocapnia as a compensation mechanism for the severe metabolic acidemia have been reported (Manohar et al. 2001).

A weakness of our study is the low number of animals used. One of our main conclusions (longer induction times should result in ameliorated blood gas status) is based on observational data, and thus causality could be reversed (i.e., animals with extreme blood gas values have a faster induction and are thus sampled earlier), although this is unlikely as it would negate a drug effect.

Our main conclusion is that induction time is an important factor to consider when analyzing blood gases after captures preceded by chase. Results of the initial blood gas analysis can be expected to differ considerably from normal but should show an improving trend in subsequent samples. Irrespective of their apparent metabolic status right after capture, khulan recover very well from anesthesia and behave normally immediately thereafter.

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