

THE PULMONARY AND METABOLIC EFFECTS OF SUSPENSION BY THE FEET COMPARED WITH LATERAL RECUMBENCY IN IMMOBILIZED BLACK RHINOCEROSSES (*DICEROS BICORNIS*) CAPTURED BY AERIAL DARTING

Robin W. Radcliffe,^{1,7} Mark Jago,^{2,6} Peter vdB Morkel,³ Estelle Morkel,³ Pierre du Preez,² Piet Beytell,² Birgit Kotting,² Bakker Manuel,² Jan Hendrik du Preez,⁴ Michele A. Miller,⁵ Julia Felipe,¹ Stephen A. Parry,¹ and Robin D. Gleed¹

¹ Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, 930 Campus Road, Ithaca, New York 14853, USA

² Namibian Ministry of Environment, Forestry and Tourism, Private Bag 13306, Windhoek, Namibia

³ Private Consultant, PO Box 260, Kakamas 8870, South Africa

⁴ Du Preez Wild, PO Box 510, Otjiwarongo 12001, Namibia

⁵ Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town 8000, South Africa

⁶ Current address: School of Veterinary Medicine, University of Namibia, Private Bag 13301, Windhoek, Namibia

⁷ Corresponding author (email: rwr32@cornell.edu)

ABSTRACT: Aerial translocation of captured black rhinoceroses (*Diceros bicornis*) has been accomplished by suspending them by their feet. We expected this posture would compromise respiratory gas exchange more than would lateral recumbency. Because white rhinoceroses (*Ceratotherium simum*) immobilized with etorphine alone are hypermetabolic, with a high rate of carbon dioxide production (VCO_2), we expected immobilized black rhinoceroses would also have a high VCO_2 . Twelve (nine male, three female; median age 8 yr old [range: 4–25]; median weight 1,137 kg [range: 804–1,234] body weight) wild black rhinoceroses were immobilized by aerial darting with etorphine and azaperone. The animals were in lateral recumbency or suspended by their feet from a crane for approximately 10 min before data were collected. Each rhinoceros received both treatments sequentially, in random order. Six were in lateral recumbency first and six were suspended first. All animals were substantially hypoxicemic and hypercapnic in both postures. When suspended by the feet, mean arterial oxygen pressure (P_aO_2) was 42 mm Hg, 4 mm Hg greater than in lateral recumbency ($P=0.030$), and arterial carbon dioxide pressure (P_aCO_2) was 52 mm Hg, 3 mm Hg less than in lateral recumbency ($P=0.016$). Tidal volume and minute ventilation were similar between postures. The mean VCO_2 was 2 mL/kg/min in both postures and was similar to, or marginally greater than, VCO_2 predicted allometrically. Suspension by the feet for 10 min did not impair pulmonary function more than did lateral recumbency and apparently augmented gas exchange to a small degree relative to lateral recumbency. The biological importance in these animals of numerically small increments in P_aO_2 and decrements in P_aCO_2 with suspension by the feet is unknown. Black rhinoceroses immobilized with etorphine and azaperone were not as hypermetabolic as were white rhinoceroses immobilized with etorphine.

Key words: Dead space, helicopter, hypercapnia, hypoxemia, oxygenation, suspension by feet, translocation.

INTRODUCTION

Poaching is a serious threat to the wild black rhinoceros (*Diceros bicornis*) in southern Africa. In addition, agricultural encroachment has reduced their range and isolated small herds, which promotes genetic homogeneity (Muya et al. 2011; Moodley et al. 2017). To minimize these adverse effects, governments, with support from nongovern-

mental organizations, actively manage the black rhinoceros population across southern Africa. Capture and subsequent translocation are a crucial part of such active management (Linklater et al. 2012). Unfortunately, the rugged terrain in which many black rhinoceroses live can make translocation by truck impractical or impossible. To circumvent ground transportation, in July 2010 the Namibian Ministry of Environment, Forestry



FIGURE 1. Helicopter-assisted aerial slinging of a black rhinoceros (*Diceros bicornis*) by its feet during translocation operations in Namibia.

and Tourism adopted the practice of airlifting immobilized black rhinoceroses by suspending them from their feet under a helicopter for periods of up to 30 min (Fig. 1). The physiological effects on the rhinoceros of such transport have not been described.

Established protocols for capture of wild black rhinoceroses use potent opioids such as

etorphine. These drugs may contribute to serious or fatal complications including hypoventilation, hypoxemia, hypercapnea, hypertension, and acidemia (Kock et al. 1990; Morkel et al. 2010; Fahlman et al. 2016). To facilitate loading for ground transportation, the opioids used for capture are usually partially antagonized, decreasing their adverse

side-effects (Miller et al. 2013; Haw et al. 2014). However, safe aerial suspension under a helicopter requires complete immobility and, therefore, partial opioid antagonism is not routinely employed in black rhinoceroses that are air-lifted.

Furthermore, posture under anesthesia affects pulmonary and cardiovascular function (Steffey et al. 1990). Immobilized black rhinoceros in sternal recumbency have significantly greater median arterial oxygen pressure (P_aO_2) than in lateral recumbency (56 mm Hg vs. 41 mm Hg, respectively), although alveolar ventilation is similar in the two postures (Radcliffe et al. 2014). Unfortunately, no physiological data are available to assess the risks for airlifted rhinoceroses suspended by their feet. A white rhinoceros (*Ceratotherium simum*) positioned in dorsal recumbency for colic surgery experienced more profound hypoxemia than did rhinoceroses in lateral recumbency (Valverde et al. 2010). Anesthetized horses (*Equus caballus*), which are closely related phylogenetically to rhinoceroses, have more venous admixture and lower arterial oxygen tension in dorsal recumbency (but not suspended) than in lateral recumbency (Nyman and Hedenstierna 1989). Although objective measurements are not available, horses suspended from a hoist by their feet appear by visual inspection to be dyspneic because the abdominal contents restrict diaphragm movement and, perhaps, are displaced cranially, thus compressing the lungs and other thoracic organs. Consequently, suspending recently captured black rhinoceroses by their feet for aerial transportation might compromise their pulmonary function at a time when they are already hypoxemic and hypercapnic.

Because there is a paucity of physiological information on airlifted rhinoceros, the first aim of this study was to collect measurements on black rhinoceroses suspended by their feet from a crane to mimic the position that they would be in while being transported under a helicopter. Specifically, we hypothesized that immobilized black rhinoceroses suspended by their feet would have higher arterial carbon dioxide pressure (P_aCO_2) and lower P_aO_2 than

they would lying on the ground in lateral recumbency.

White rhinoceroses immobilized with etorphine have oxygen consumption and carbon dioxide production (VCO_2) greater than might be expected (Buss et al. 2018); this could be caused by increased skeletal muscle activity (de Lange et al. 2017). It is possible that this hypermetabolic state contributes to the hypoxemia, hypercapnea, hypertension, and acidemia observed when white rhinoceroses are immobilized with etorphine. No similar metabolic data are available for black rhinoceroses. However, the technique used for measuring ventilation in the field allows calculation of the VCO_2 . Because a hypermetabolic state is reported in white rhinoceroses after etorphine (Buss et al. 2018; Boesch 2020), the second aim of this study was to assess whether black rhinoceroses immobilized with etorphine and azaperone were in a similar hypermetabolic state. To test this, we hypothesized that immobilized black rhinoceroses would have a greater VCO_2 than that predicted allometrically.

MATERIALS AND METHODS

Study site and subjects

The research protocol was approved by Cornell University Institutional Animal Care and Use Committee (protocol no. 2006-0170) and by Namibian Ministry of Environment, Forestry and Tourism. From 7–24 April 2015, 39 rhinoceroses were immobilized in Waterburg National Park (20°30'52''S, 17°14'45''E) primarily for management procedures unrelated to this study. Twelve of these animals were subadult (age range: 4–5 yr old; $n=3$) or adult (range: 7–25 yr old; $n=9$) black rhinoceroses and were enrolled in the study because they were in locations accessible by a truck carrying a crane for hoisting. Age was estimated based on tooth emergence and wear following Hitchins (1978). These 12 subjects were immobilized via remote intramuscular injection by darting (Cap-Chur Equipment, Powder Springs, Georgia, USA) from a helicopter using etorphine hydrochloride 5.0 mg (range: 4.0–5.5; M99, Novartis, Kempton Park, South Africa), azaperone 60 mg (Stressnil, Janssen Pharmaceutical Ltd., Halfway House, South Africa), and hyaluronidase 2,500 IU (Hyalase, Kyron Laboratories, Benrose, South Africa). After data collection and completion of management procedures,



FIGURE 2. Hanging posture of a black rhinoceros (*Dicerus bicornis*) using a crane on a flatbed truck to simulate aerial suspension under a helicopter in Namibia.

etorphine was antagonized with either diprenorphine (12 mg total dose; $n=4$) or naltrexone (100 mg total dose; $n=8$) by intravenous injection in an auricular vein.

Measurements

Observations were made in two sequential phases on each animal: lateral recumbency on the ground and suspended by all four feet with the head clear of the ground using a crane on a flatbed truck to simulate aerial suspension by helicopter (Fig. 2). The order of the phases was assigned randomly so that six animals were in lateral recumbency first and six were suspended first. When the ground crew had reached the rhinoceros and the animal could be approached safely, it was manipulated into lateral recumbency (if necessary); once the investigators reached the animal it was either left in lateral recumbency or suspended, depending on its assigned initial posture. Previously collected data suggested any differences in arterial blood gas tensions or other ventilatory variables between left and right lateral recumbency were much less than 10% and smaller than differences between lateral and sternal recumbency. Therefore, animals assigned to lateral recumbency were allowed to stay on the side they adopted when they first went down

(Radcliffe et al. 2014). Two sets of measurements were taken; one after animals had been in the first assigned posture for approximately 10 min after the investigating team reached the animal and another after animals had been in the second assigned posture for approximately 10 min.

Ambient temperature and atmospheric pressure (P_{atm}) were measured at the capture site (Kestrel® 4300 Weather Meter, Nielsen-Kellerman, Boothwyn, Pennsylvania, USA). Cuffed tubes were placed inside each nostril (26 mm internal diameter; SurgiVet, Smiths Medical North America, Dublin, Ohio, USA) and attached to a purpose-built system of polyvinyl chloride tubes, one-way valves, and two 200-L collection bags (Radcliffe et al. 2014). The collection bags were shaded from the sun with a beach umbrella. Expired gas was collected for 2 min while the respiratory rate was counted. At the end of this period, the collecting system was disconnected from the animal and sealed. During expired gas collection, a sampling tube in the nasal passage (VitaLine™ H Set, Oridion Capnography, Bedford, Massachusetts, USA) was attached to a capnograph designed for side-stream sampling (Microcap® Plus, Oridion Capnography) to measure end-tidal partial pressure of carbon dioxide ($P_{E}CO_2$). Mixed expired $P_{E}CO_2$ was measured from a small volume aspirated from the collection

bags immediately after collection was complete, using the same device. The expired gas volume was measured by completely expressing the gas from the collection bags through a spirometer (Mark 8 Wright's Respirometer, Grace Medical Inc., Kennesaw, Georgia, USA) that was calibrated (RT-200 Calibration Analyzer, Timeter Instrument Corp., St. Louis, Missouri, USA). The temperature of the gas leaving the spirometer was measured with the Kestrel weather meter. Study animals were weighed using a spring scale that was attached to the crane (Dillon ED Junior scale; Dillon Force Measurement Equipment; Fairmont, Minnesota, USA). Rectal temperature was measured with a thermistor (Fluke Corporation, American Fork, Utah, USA) during collection of the expired gas. Blood was collected anaerobically from an auricular artery toward the end of gas collection over at least one respiratory cycle using a vented syringe (Smiths Medical ProVent* Plus Arterial Blood Gas Sampling Kit, Fisher HealthCare, Houston, Texas, USA). Samples were introduced immediately into a measuring device (i-STAT CG4+ cartridges and VetScan i-STAT® 1, Handheld Clinical Analyzer, Abbott Laboratories, Abbott Park, Illinois, USA). Values for P_{aO_2} , P_aCO_2 , arterial pH (pH_a), and lactate were measured, while base excess of the extracellular fluid and saturation of arterial hemoglobin with oxygen (S_aO_2) were calculated by the analyzer software. Values for P_aO_2 , P_aCO_2 , and pH_a were corrected to rectal temperature by the analyzer software.

Analyses

Minimum sample size was estimated a priori using data from recently captured black rhinoceroses in lateral recumbency where the mean P_aCO_2 was 50 mm Hg (4 SD; Radcliffe et al. 2014). Based on these data, seven individuals would be enough to detect a 5-mm Hg (10% difference (two-tailed) in P_aCO_2 assuming paired comparison, power of 0.9, and alpha of 0.05. Alveolar oxygen pressure (P_{AO_2}) was calculated from the alveolar gas equation (eq. 1):

$$P_{AO_2} = 0.21(P_{atm} - P_{H_2O}) - (P_aCO_2/R), \quad (1)$$

where P_{H_2O} was the water vapor pressure at the rectal temperature and R was the respiratory quotient, the ratio of carbon dioxide produced to oxygen consumed (assumed to be 0.8). Alveolar to arterial oxygen pressure difference ($P_{(A-a)O_2}$) and arterial to end-tidal carbon dioxide pressure difference ($P_{(a-E)CO_2}$) were then calculated.

Tidal volume (VT) was calculated by dividing expired minute ventilation (VE) by the breathing rate (fR). Both VE and VT are reported after normalization to body weight and after correction

to rectal temperature and atmospheric pressure and for saturation with water vapor at body temperature (Lumb 2000).

Total physiological dead space ratio (VD/VT) was calculated by applying the Enghoff modification of the Bohr equation (eq. 2):

$$VD/VT = (P_aCO_2 - P_ECO_2)/P_aCO_2 \quad (2)$$

Rate of production of carbon dioxide (VCO_2) was calculated using eq. 3:

$$VCO_2 = VE_{STPD} \cdot P_ECO_2 / (P_{atm} - P_{H_2Oamb}), \quad (3)$$

where VE_{STPD} was minute ventilation corrected to standard temperature and pressure (STPD) and P_{H_2Oatm} was the saturated water vapor pressure at ambient temperature (Lumb 2000). The VCO_2 is reported at STPD and after normalization to body weight.

An allometric equation was used to estimate normal resting oxygen consumption (V_{alloO_2}) values for each animal (Stahl 1967; Schmidt-Nielsen 1984). Assuming a steady state, substituting V_{alloO_2} in equation 4 permitted an allometric estimate of carbon dioxide production (V_{alloCO_2}):

$$V_{alloCO_2} = V_{alloO_2} \cdot R. \quad (4)$$

Because the value of R is likely to be in the range 0.8 to 1.0, two allometric estimates of V_{alloCO_2} were calculated from each V_{alloO_2} value; one assuming R was 0.8 ($V_{allo0.8CO_2}$) and the other assuming it was 1.0 ($V_{allo1.0CO_2}$). For comparison with measured values, allometric equations were applied to median body weight to obtain estimates of normal values for tidal volume (V_{alloT}), minute ventilation (V_{alloE}), and breathing rate (f_{alloR} ; Stahl 1967; Schmidt-Nielsen 1984).

Statistical significance was determined as $P < 0.05$. The responses that were considered for primary statistical analysis were: P_aO_2 , P_aCO_2 , pH_a , S_aO_2 , P_{AO_2} , base excess of the extracellular fluid, lactate, VT, fR , VE, P_ECO_2 , $P_{(a-E)CO_2}$, VD/VT, VCO_2 , and $P_{(A-a)O_2}$. Linear mixed effect models were run for each response with fixed effects of posture and treatment order and a random effect of rhinoceros identification number (JMP Pro 14, 100 SAS Campus Drive, Cary, North Carolina, USA). Treatment order was included as a fixed effect to control for the cases that were assigned to lateral recumbency first; unlike the animals that were suspended first, these animals experienced no change in posture before the first data were collected; they might also have been in lateral recumbency for longer because of time elapsed in lateral recumbency before the investigators arrived at the site of the observations. For responses where treatment

TABLE 1. Descriptors for 12 immobilized wild black rhinoceros (*Diceros bicornis*) in Namibia during investigations comparing the pulmonary physiology of lateral position with aerial suspension by the feet. Each rhinoceros received both treatments sequentially, in random order. Data are collated by whichever treatment was assigned first; either lateral recumbency or suspension by the feet. Age and weight are expressed as median (minimum–maximum).

	Lateral recumbency first (n=6)		Suspended by the feet first (n=6)	
	n or median	Range ^a	n or median	Range ^a
Female (n)	2	NA	1	NA
Male (n)	4	NA	5	NA
Subadult (n)	2	NA	1	NA
Adult (n)	4	NA	5	NA
Median age (yr)	9	4–25	8 ^b	5–20
Median weight (kg)	1,077	804–1,172	1,138	940–1,234

^a NA = not applicable.

^b n=5 (one adult not aged by dentition).

order had a significant effect, the data were subdivided by treatment order and the significance of differences between values obtained in lateral recumbency first and those obtained in lateral recumbency second were measured using a two-tailed, unpaired *t*-test (GraphPad Prism 6, La Jolla, California, USA); the significance of differences between values in suspension first and in suspension second were measured likewise. The significance of differences between V_{CO_2} and $V_{alve}CO_2$ were measured with the above two-tailed, unpaired *t*-test. Data that underwent statistical analysis are reported as mean (SD); unless otherwise stated, data that did not undergo statistical analysis are reported as median (range: minimum–maximum).

RESULTS

The median weight of nine male and three female rhinoceroses was 1,137 kg (range: 804–1,234) and the animals were a median age of 8 yr old (range: 4–25; Table 1). The median doses of etorphine, azaperone, and hyaluronidase used for darting were 4.6 µg/kg (range: 4.2–5.4), 53 µg/kg (range: 49–75), and 2.2 IU/kg (range: 2.0–3.1), respectively. The animals became laterally recumbent 4.4 min (range: 1.2–6.7) after being darted. The median ambient temperature was 27.8 C (range: 21.4–27.8) and the median atmospheric pressure was 629 mm Hg (range: 627–632). The median rectal temperature of the rhinoceroses in lateral recumbency was 37.6 C (range: 36.6–38.7) and 37.7 C (range: 36.8–38.7)

when they were suspended by their feet (Table 2). Measurements were completed within 55 min (range: 44–71) of the animal becoming recumbent. All animals recovered uneventfully.

Regardless of posture, the rhinoceroses were substantially hypoxemic and hypercapnic; no individual rhinoceros had $P_{aO_2} > 47$ mm Hg or $P_{aCO_2} < 50$ mm Hg (Table 2). Two of 12 rhinoceroses in lateral recumbency had $pH_a > 7.35$, whereas five of 12 suspended animals had $pH_a > 7.35$. Base excess was in the range of 7 to –2 mmol/L and lactate was between 0.9 and 4.8 mmol/L.

When rhinoceros were suspended by their feet, there were significant differences in P_{aO_2} , S_{aO_2} , pH_a , P_{aCO_2} , $P_{(a-E)CO_2}$, VD/VT , and P_{AO_2} when compared to animals in lateral recumbency (Tables 2, 3). In suspended animals, mean P_{aO_2} was 4 mm Hg greater, mean S_{aO_2} was 8% greater, and mean pH_a was greater compared with lateral recumbency (7.34 vs. 7.32; Table 2). The mean P_{aCO_2} was 3 mm Hg less when rhinoceroses were suspended (Table 2) than it was when they were in lateral recumbency. Mean $P_{(a-E)CO_2}$ and VD/VT were 4 mm Hg and 4% less, respectively, when the animals were suspended (Table 3) compared with lateral recumbency. Mean P_{AO_2} was 4 mm Hg greater when the rhinoceroses were suspended (Table 3). Base excess, lactate, VT , fR , VE , V_{CO_2} ,

TABLE 2. Median (minimum–maximum) rectal temperature and mean (SD) arterial blood gas tensions and acid-base values from 12 immobilized wild black rhinoceroses (*Diceros bicornis*) in Namibia during investigations comparing the pulmonary physiology of lateral position with aerial suspension by the feet. Animals were in lateral recumbency or suspended by the feet for approximately 10 min before data collection. Each rhinoceros received both treatments sequentially, in random order. The alpha value for comparisons was 0.05.^a

Measurements	Lateral recumbency (n=12)		Suspended by the feet (n=12)		P
	Median or mean	SD or range	Median or mean	SD or range	
Median rectal temperature (C; range)	37.6	36.6–38.7	37.7	36.8–38.7	NE
Mean P _a O ₂ (mm Hg)	38	5.6	42	3.7	0.030*
Mean S _a O ₂ (%)	63	9.9	71	6.7	0.025*
pH _a	7.32	0.03	7.34	0.03	0.011*
P _a CO ₂ (mm Hg)	55	5.4	52	4.3	0.016*
Base excess of extracellular fluid (mmol/L)	2.3	1.72	2.1	2.23	0.536
Lactate (mmol/L)	2.1	1.08	2.0	0.97	0.425

^a P_aO₂ = arterial oxygen pressure; S_aO₂ = saturation of arterial hemoglobin with oxygen; P_aCO₂ = arterial carbon dioxide pressure; NE = not evaluated.

* = significantly different.

and P_(A-a)O₂ were not significantly different between the two treatments (Tables 2, 3).

Only P_aCO₂ and P_AO₂ were significantly affected by the order of treatment. Mean P_aCO₂ was 6 mm Hg greater and mean P_AO₂ was 8 mm Hg less in the animals that were in lateral recumbency first compared with those when they were in lateral recumbency second; no such effects of treatment order were apparent in the animals when they were suspended by the feet (Table 4). Mean time

in posture before data collection was 14 min longer for those animals that were in lateral recumbency first as compared with those that were in lateral recumbency second; no such effect of treatment order was apparent in the animals when they were suspended (Table 4). When suspension by the feet was followed by a move to the lateral position, the mean P_aCO₂ increased, while mean P_aO₂ and pH_a declined, in five of six animals, whereas a move from lateral recumbency to suspension

TABLE 3. Mean (SD) values from 12 immobilized wild black rhinoceroses (*Diceros bicornis*) in Namibia during investigations comparing the pulmonary physiology of lateral position with aerial suspension by the feet. Animals were in lateral recumbency or suspended by the feet for approximately 10 min before data collection. Each rhinoceros received both treatments sequentially, in random order. The alpha value for comparisons was 0.05.^a

Measurements	Lateral recumbency (n=12)	Suspended by the feet (n=12)	P
Tidal volume (mL/kg)	11 (2.9)	11 (1.7)	0.783
Breathing rate (breaths/min)	5.6 (1.2)	5.5 (1.1)	0.792
Minute ventilation (mL/kg/min)	59 (2.6)	59 (1.2)	0.803
P _E CO ₂ (mm Hg)	39 (5.5)	39 (4.7)	0.626
P _(a-E) CO ₂ (mm Hg)	16 (3.1)	12 (3.2)	0.002*
Enghoff dead space fraction (%)	56 (4)	52 (4)	0.005*
VCO ₂ (mL/kg/min)	2.3 (0.44)	2.4 (0.40)	0.614
P _A O ₂ (mm Hg)	53 (6.6)	57 (5.3)	0.017*
P _(A-a) O ₂ (mm Hg)	15 (5.3)	15 (2.6)	0.927

^a P_ECO₂ = end-tidal pressure of carbon dioxide; P_(a-E)CO₂ = arterial to end-tidal carbon dioxide pressure difference; VCO₂ = rate of carbon dioxide production; P_AO₂ = alveolar oxygen pressure; P_(A-a)O₂ = alveolar to arterial oxygen pressure difference.

* = significantly different.

TABLE 4. Mean (SD) values from 12 immobilized black rhinoceroses (*Diceros bicornis*) in Namibia during investigations comparing the physiology of lateral position with aerial suspension by the feet. The animals were in lateral recumbency or suspended by their feet before data were collected. Each rhinoceros received both treatments sequentially, in random order. These data are subdivided by the order (first or second) in which each treatment (lateral recumbency or suspension by the feet) was applied. P_aCO_2 (arterial carbon dioxide pressure) and P_aO_2 (alveolar oxygen pressure) are the only parameters where the order of treatment had a significant effect. The alpha value for comparisons was 0.05.

Order of treatment	Lateral first (n=6)	Lateral second (n=6)	P	Suspended first (n=6)	Suspended second (n=6)	P
P_aCO_2 (mm Hg)	58 (4.2)	52 (4.5)	0.025*	53 (2.6)	50 (5.1)	0.167
P_aO_2 (mm Hg)	49 (5.2)	57 (5.5)	0.025*	55 (3.2)	59 (6.3)	0.177
Time to collect data (min) ^a	26 (8.9)	12 (1.2)	0.004*	14 (2.6)	12 (4.0)	0.414

^a Time to collect data = time in posture before data were collected.

* = significant difference depending on whether the animals were in lateral recumbency for the first treatment or for the second treatment.

was followed by corresponding changes in the opposite direction in four of six animals.

Mean calculated allometric estimates of carbon dioxide production were 1.7 mL/kg/min (0.05) for $V_{allo0.8}CO_2$ and 2.2 mL/kg/min (0.07) for $V_{allo1.0}CO_2$. Mean VCO_2 measured in lateral recumbency was 2.3 mL/kg/min (0.44), which was 0.5 mL/kg/min greater ($P=0.0001$) than $V_{allo0.8}CO_2$, but mean VCO_2 was not significantly different from $V_{allo1.0}CO_2$ ($P=0.238$). The allometrically predicted pulmonary variables, $V_{allo}T$, $V_{allo}E$, and $f_{allo}R$, were 10 mL/kg, 94 mL/kg/min, and 8.7 breaths per min, respectively (Schmidt-Nielsen 1984). By inspection, measured VT was similar to $V_{allo}T$, but measured VE and fR were much less than the corresponding allometric normal values (Table 3).

DISCUSSION

Our findings allowed us to reject our first hypothesis and showed that suspending immobilized black rhinoceros by their feet for 10 min did not impair pulmonary function more than did lateral recumbency. All immobilized black rhinoceroses in our study were severely hypoxemic and hypercapnic regardless of whether they were suspended by their feet or lying in lateral recumbency. The severity of the hypoxemia and hypercapnia concurs with observations that others have made in free-ranging black rhinoceroses captured using

potent opioids (Kock et al. 1990; Fahlman et al. 2016).

In this study, suspension by the feet was actually associated with very slightly better respiratory gas exchange, as suggested by numerically small, but statistically significant, greater mean P_aO_2 and lower mean P_aCO_2 in this position. Although improvements in arterial blood gases are numerically slight, the increment in mean P_aO_2 and the decrement in mean P_aCO_2 associated with suspension by the feet may have biological and clinical significance in animals such as these that are already severely hypoxemic and hypercapnic. These improvements with suspension may be especially critical because translocation of rhinoceroses exacerbates water loss, mobilizes energy reserves, damages muscles, and leads to oxidative stress (Pohlin et al. 2020). Because VT, VE, and fR values were similar in the two test postures, the slightly smaller mean P_aCO_2 in suspended rhinoceroses may be attributable to greater alveolar ventilation and correspondingly smaller dead space ventilation in this posture compared with lateral recumbency (Radcliffe et al. 2014). The black rhinoceroses had slightly greater pH_a when they were suspended compared with lateral recumbency. Because base excess and lactate were not significantly different between the two postures, the greater pH_a was most likely due to respiratory compensation via improved alveo-

lar ventilation and lesser $P_a\text{CO}_2$ when they were suspended.

Suspension resulted in a slightly greater mean $P_a\text{O}_2$ compared with lateral recumbency. However, because $P_{(A-a)}\text{O}_2$ was not significantly different between the postures, the greater mean $P_a\text{O}_2$ did not appear to be caused by less venous admixture in suspended rhinoceroses. Therefore the greater $P_a\text{O}_2$ with suspension might be due to the slightly greater $P_A\text{O}_2$ in suspended animals, which is consistent with the improved alveolar ventilation postulated above for animals in suspension. The $S_a\text{O}_2$ was 8% greater when the rhinoceroses were suspended. Although our data do not allow us to confirm it, this small increment in $S_a\text{O}_2$ might make a biologically important contribution to oxygen availability in these severely hypoxemic animals.

Because measured VCO_2 was 35% greater than $V_{\text{allo}0.5}\text{CO}_2$ but was not significantly different from $V_{\text{allo}1.0}\text{CO}_2$, our data must be considered equivocal regarding the postulated hypermetabolic effect of immobilization in black rhinoceros (our second hypothesis). In white rhinoceros, VCO_2 was 4.2 (1.1), 3.9 (0.7), and 4.6 mL/kg/min (0.5) at 30, 40, and 50 min, respectively, after being given etorphine alone; these values are consistent with a markedly hypermetabolic state in white rhinoceroses (Boesch 2020). Our measured values for VCO_2 in immobilized black rhinoceros are considerably less than those reported in white rhinoceros after etorphine. This difference in VCO_2 between species might be due to black rhinoceros being inherently less susceptible than white rhinoceros to the hypermetabolic effects of etorphine. Although it is possible that the azaperone tranquilizer coadministered with etorphine in the black rhinoceroses muted an increase in metabolic rate from the etorphine, others have reported no relief from hypoxemia when azaperone is given with etorphine to white rhinoceroses (de Lange et al. 2017). The measured VCO_2 reported here in black rhinoceroses reinforces clinical experience suggesting that there are differences between the two species of African rhinoceros in the way they respond

to immobilization with drug combinations that include μ -opioid agonists.

Hypercapnia (mean $P_a\text{CO}_2 > 50$ mm Hg) observed in rhinoceroses in both positions is compatible with impaired alveolar ventilation. Measured VE in these rhinoceroses was 63% of the allometrically predicted normal value and consistent with hypoventilation. Measured VT was similar to that predicted allometrically; however, measured fR was 63% of that predicted for normal awake rhinoceroses (Citino and Bush 2007), suggesting that the hypoventilation is due principally to inhibition of breathing pattern generation. In other species, μ -opioids primarily inhibit respiratory pattern generation rather than tidal volume, thus it is likely that the hypoventilation in our rhinoceroses was attributable to the etorphine component of the immobilizing drug combination resetting the rhinoceros' carbon dioxide-sensing mechanism and slowing their breathing rate (Lalley 2003).

The randomly assigned treatments in a paired cross-over design aided detection of small significant differences. However, this model produced a limitation to the study, particularly related to the six animals that were in lateral recumbency first. Because all 12 animals went into lateral recumbency after darting, the six animals assigned to be in lateral recumbency first were not suspended at all before the first data were collected, whereas the other six animals were suspended before any data were collected. Also the six animals assigned to be in lateral recumbency first were in lateral recumbency for longer than the corresponding cohort that was in lateral recumbency as their second treatment. Only $P_a\text{CO}_2$ and $P_A\text{O}_2$ were affected by order of treatment, such that those animals that were in lateral recumbency first had higher $P_a\text{CO}_2$ and lower $P_A\text{O}_2$. This observation might be due to prolonged time in lateral recumbency without autonomic perturbation associated with posture change before the observations were made or to some other factor.

Dead space fraction in laterally recumbent rhinoceroses in our study was similar to that

reported previously with the same equipment in black rhinoceroses (Radcliffe et al. 2014). Such a large dead space fraction is unusual. Because the Enghoff method used here substitutes $P_a\text{CO}_2$ for alveolar PCO_2 in the Bohr equation, it is likely to overestimate dead space when intrapulmonary shunt is large or there are large areas of lung with low V/Q ratio (Wagner et al. 2008). Although shunt fraction was not measured here, the low $P_a\text{O}_2$ and large $P_{(A-a)}\text{O}_2$ suggest that shunt fraction was considerable in these rhinoceroses, making such overestimation of total respiratory dead space likely. A more accurate measure of dead space uses volumetric capnography, which requires simultaneous measurement of expired volume and expired carbon dioxide; unfortunately, the equipment for this was not available in the field. Enghoff dead space, as measured here, should be viewed as a composite of dead space ventilation plus intrapulmonary venous admixture (Suarez-Sipmann et al. 2013). That being the case, values in excess of 50% for Enghoff dead space, as we report, might be evidence of severe, but nonspecific, impairment of pulmonary gas exchange.

These experiments suggest that the pulmonary system of immobilized black rhinoceros is no more compromised by suspension by the feet for 10 min than it is by lying in lateral recumbency. However, because helicopter translocation of black rhinoceroses usually takes longer than 10 min, future experiments should be directed to studying how longer periods of suspension by the feet influence the severe hypoxia and hypercapnia observed here. Such studies should also determine whether the small improvements in $P_a\text{O}_2$ and $P_a\text{CO}_2$ we observed with suspension are consistent.

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