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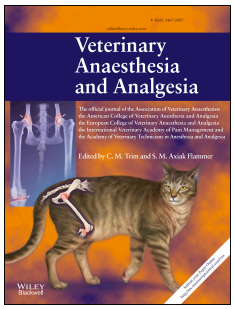

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RESEARCH PAPER

Evaluation of BAM (butorhpanol-azaperone-medetomidine) in captive African lion immobilization (*Panthera leo*)

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Running head: BAM in African lion immobilization

Abstract

Objective The combination of butorphanol, azaperone and medetomidine (BAM) with subsequent antagonism by naltrexone-yohimbine or naltrexone-atipamezole was evaluated for reversible immobilization of captive African lions (*Panthea leo*).

Study design Prospective, clinical trial.

Animals Twenty lions, 11 males and nine females, weighing 38-284 kg were immobilized in South Africa.

Methods BAM volume dose rate administered was 0.005 - 0.008 mL kg^{-1} (0.6 mL 100 kg^{-1}). Physiologic variables were recorded every 5 minutes. Four arterial blood samples were collected from all animals at 20, 30, 40 and 50 minutes after immobilization for analysis of blood gases and acid-base status.

Results The actual doses administered were as follows: butorphanol (0.18 ± 0.03 mg kg^{-1}), azaperone (0.07 ± 0.01 mg kg^{-1}) and medetomidine (0.07 ± 0.01 mg kg^{-1}). The inductions were calm and smooth and induction time ranged from four to ten minutes (7 ± 2 minutes). The amount of time needed to work with each lion was 70 minutes and no additional drug doses were needed. Heart rate (40 ± 8 beats $minute^{-1}$) and respiratory frequency (15 ± 4 breaths $minute^{-1}$) were stable throughout immobilization. Mean arterial blood pressure of all the animals was stable but elevated (142 ± 16 mmHg). Rectal temperature slightly increased over time but remained within an acceptable range. The recovery time was significantly shorter when using naltrexone and atipamezole (9 ± 1 minutes) compared to using naltrexone and yohimbine (22 ± 7 minutes).

Conclusion and clinical relevance The BAM combination proved to be reliable for general veterinary anaesthesia in lions. During anaesthesia, minor veterinary

procedures such a blood collection, intubation, vaccination and collaring could safely be performed with no additional dosing required.

Keywords azaperone, BAM, butorphanol, lion medetomidine

Introduction

African lions (*Panthera leo*) are often immobilized for routine procedures such as microchipping, collaring, disease prevention and medical treatment. These immobilizations require the use of drugs that are safe, not only for the animals but also for the people working with them (i.e. induce a deep plane of anaesthesia) since lions are notoriously aggressive and dangerous.

Traditionally, dissociative anaesthetics (ketamine and tiletamine) combined with relatively small concentrations of sedative and tranquilizing medications (xylazine, medetomidine, detomidine) are applied for the anaesthesia of lions (Fahlman et al. 2005; Jacquiler et al. 2006; Fyumagwa et al. 2012). Combinations of ketamine-xylazine and tiletamine-zolazepam-medetomidine are considered to be the most suitable combinations (Herbst et al. 1985; Fahlman et al. 2005; Jacquiler et al. 2006). The application of all the above-mentioned combinations has both advantages and disadvantages (Herbst et al. 1985; Tomizawa et al. 1997; Fahlman et al. 2005; Jacquiler et al. 2006; Fyumagwa et al. 2012; Kreeger & Arnemo 2012). More recently, a combination of butorphanol, medetomidine and midazolam (BMM) has been successfully used in free-ranging lions for a period of 45 minutes (Wenger et al. 2010).

BAM (the combination of butorphanol-azaperone-medetomidine) as described in this article, is a dry mixture, containing 300 mg of butorphanol tartrate, 100 mg of azaperone tartrate, and 120 mg of medetomidine hydrochloride. The use of this combination has been reported in species such as white-tailed deer (*Odocoileus virginianus*) (Mich et al. 2008; Miller et al. 2009; Siegal-Willott et al. 2009), rocky mountain elk (*Cervus elaphus nelsoni*) (Wolfe et al. 2014), Nubian ibex (*Capra nubiana*) (Lapid & Shilo-Benjamini 2015), bighorn sheep (*Ovis canadensis*) (Smith et al. 2014) and black bear (*Ursus americanus*) (Wolfe et al. 2008). It has been reported to produce reversible anaesthesia without hyperthermia and good analgesia. In addition, it can be delivered with a low-volume dart (Lance 2008). One disadvantage noted in cervids is that this combination may result in significant hypoxia and oxygen supplementation is recommended (Mich et al. 2008; Miller et al. 2009; Siegal-Willott et al. 2009).

To our knowledge, this is the first time that this combination has been used as a ready-made medication for the immobilization of a felid species, specifically lions. The aims of this study were to acquire comprehensive and reliable monitoring data as well as develop, explore, and describe the use of BAM for the immobilization of captive African lions.

Materials and methods

Animals, medications and delivery methods

Twenty lions (11 males and nine females) were immobilized with BAM on the Lechwe Lodge private game farm in the Free State province [August 2014 (13 lions)] and at Mohololoholo Wildlife Rehabilitation Center in the Limpopo province [September 2015 (seven lions)] in the Republic of South Africa. The animals were

immobilized for clinical examination, GPS collaring, deworming, contraceptive implantation and genetic material collection.

The BAM solution (BAM, Wildlife Pharmaceuticals South Africa (Pty) Ltd., South Africa) was prepared by dissolving one vial containing 300 mg of butorphanol tartrate, 100 mg of azaperone tartrate and 120 mg of medetomidine hydrochloride in 10 mL of sterile water for injection (Pharma-Q water for injection, Pharma-Q, South Africa). Each mL of the solution contained 30 mg of butorphanol, 12 mg of azaperone, and 12 mg of medetomidine. The individual doses for the combination were calculated based on commonly accepted recommendations for use of the above mentioned active agents as well as the data acquired during previous immobilizations of lions conducted by the authors. The body weight of the animals was estimated based on visual parameters

All the animals were immobilized between 6:00 to 13:00 and 15:00 to 17:00 hours in order to avoid high, midday environmental temperatures. The air temperature ranged from 4.0 to 33.4°C. The elevation above mean sea level (AMSL) was 1399 m at Lechwe Lodge and about 520 m at Mohololoholo Rehabilitation Center.

A cartridge fired projector (Pneu-Dart Model 389, Wildlife Pharmaceuticals (Pty) Ltd.) was used to deliver the anaesthetic. Darts (Pneu-Dart Type ‘C’) with a volume of 1-3 mL and a length of 0.75-1.5 inches and 13-16 gauge needles with wire barbs were used (Wildlife Pharmaceuticals (Pty) Ltd.). Remote darting was done in enclosures from inside a vehicle or on foot from distances ranging from 3 to 21 meters. Distance was measured using Leupold RX-1000i rangefinder (Leupold & Stevens, OR, USA). The injections were administered into the femoral muscles.

For reversing the effect of the medetomidine in 13 cases, a formulation of 6.25 mg mL^{-1} yohimbine hydrochloride at a dose rate of 0.2 mg kg^{-1} body weight was

used. In 7 cases, atipamezole (Antisedan 5 mg mL^{-1}; Orion Pharma, Finland) at five times the medetomidine dose in mg was used. Naltrexone hydrochloride (Trexonil 50 mg ml^{-1}; Wildlife Pharmaceuticals (Pty) Ltd.) was used to reverse butorphanol at one time (mg to mg) the actual butorphanol dose. All the injections were given intramuscularly (IM).

Monitoring and manipulations of animals

Two stages of induction were timed: stage I – from time of the darting till the first signs of sedation, including open mouth, ataxic gait and lowering of the head; stage II – from the injection time till sternal or lateral recumbency. Once the animals reached lateral recumbency, they were blindfolded and transported from the enclosure to a controlled environment, no further than 100 m from the enclosure, where monitoring could be performed. All animals were intubated using endotracheal tubes (Jorgensen Labs, CO, USA) 16-24 mm in diameter. Every 5 minutes, beginning at 15-20 minutes after darting, monitoring of physiological parameters [heart rate (HR), respiratory frequency (f_R), oxygen saturation (SpO_2), end-tidal carbon dioxide ($P_E´CO_2$), non-invasive blood pressure (NIBP) and body temperature (BT)] was conducted using a veterinary monitor (Capnovet Deluxe Multiparameter Monitor; Eickemeyer, Germany). Auscultation with a stethoscope (3M Littmann Classic II S.E. Stethoscope; 3M United States, USA) was performed every 5 minutes for the entire period of immobilization. The level of muscle relaxation was assessed based on the general muscle tone and position of the lower jaw using a 3-point scale. Level 1 indicated the absence of muscle tone, level 2 – a light tone and level 3 – a strongly marked tone. Capillary refill time and palpebral reflex were additionally registered.

Arterial blood samples were collected from the femoral or median caudal artery at 20, 30, 40 and 50 minutes after darting. The samples were immediately analysed using a portable analyser (i-STAT1 Portable Clinical Analyzer; Abaxis, CA USA) and cartridges (i-STAT cartridges CG4+ & CHEM8+; Abaxis). Variables measured included pH, partial pressure of arterial oxygen (PaO_2), partial pressure of carbon dioxide ($PaCO_2$), lactate, haematocrit, sodium, potassium, chlorine, urea, creatinine, glucose, and ionized calcium levels. Actual base excess, actual bicarbonate, arterial haemoglobin, oxygen saturation, and haemoglobin were calculated from the measured values.

The animals were extubated 65-70 minutes after the beginning of immobilization provided a strongly marked palpebral reflex was observed. Following extubation, all animals were weighed using a portable scale (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd. Canada) and transported back to the enclosure by vehicle. In the enclosure, the lions were placed in lateral position on the ground. Antagonists were then injected IM into the femoral muscle region. The following stages of recovery were recorded: time elapsed from injection till the first signs of recovery, including eye blinking time to head lifting, time to standing and time to fully coordinated movement (i.e. full recovery).

Statistical analysis

For exploring the anaesthetic dosage effect on the lions' HR, f_R, SBP, DBP, MBP, SpO_2, $P_E´CO_2$, PaO_2 and $PaCO_2$, the area under the curve (AUC) was calculated using a trapezoid method for every measurement for the immobilization period (60 minutes). Linear regression models were used with the mean AUC as the response variable. The exact anaesthetic dosage used (calculated after weighing of immobilized lions) was divided into 3-levels (low: 0.52-0.56 mL 100 kg^{-1}; n = 6, medium: 0.59-

0.64 mL 100 kg^{-1}; *n* = 8 and high: 0.66-0.83 mL 100 kg^{-1}; *n* = 6) grouping variable, were included as an explanatory variable. Age was divided into 2 levels (sub-adults; *n* = 9 and adults; *n* = 11) as the grouping variable and sex (males; *n* = 11 and females; *n* = 9) and their interaction with dose groups were added into every model. The model's assumptions were verified by scatter and normality plots of standardized residuals.

For comparing recovery time between different antidote groups (atipamezole; *n* = 7 and yohimbine hydrochloride; *n* = 13) a nonparametric Mann-Whitney test was used. For linear regression models and Mann-Whitney test, STATA 13.0 software (Stata Corporation, TX, USA) was used.

General linear mixed models (GLMM) were used to explore the overall time trend in lactate, arterial blood pH and body temperature and differences in time trend between the dosage groups. Lions were included as random intercepts and polynomials of time (minutes), with interactions with the dosage group added as fixed effects in increasing order. The overall time trend differences between groups were tested with the F-test. Isotropic spatial exponential covariance structure was used for modelling serial correlations of repeated measurements at the within-lion level in all models. Initially gender and age group were included as fixed factors in all models. A backward elimination procedure was performed for the final models. The NLME package (Pinheiro J, Bates D, Debroy, Sarhar D: Linear and nonlinear mixed effect models. R package version 3.1-73 2006) with statistical software R 3.2.2 (R-soft. R Development Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria; 2006) was used for fitting these GLMM models.

All fitted model assumptions were verified by scatter and normality plots of standardized residuals. *P*-values of ≤ 0.05 were considered statistically significant. Data are reported as mean ± standard deviation (range).

Results

Data from 11 male lions weighing 166 ± 78 (38-284) kg and from nine female animals weighing 116 ± 29 (72-162) kg were used in this study. In all 20 animals, immobilization occurred after a single injection of BAM. There was no need for additional injections to achieve immobilization. The following dose rates were used: BAM volume dose rate range was 0.005 - 0.008 mL kg^{-1} (0.006 ± 0.001 mL kg^{-1} or 0.6 mL 100 kg^{-1}). Total dose ranged from 0.3 to 1.6 mL. The actual doses were as follows: butorphanol (0.18 ± 0.03 g kg^{-1}), azaperone (0.07 ± 0.01 mg kg^{-1}) and medetomidine (0.07 ± 0.01 mg kg^{-1}). The first sign of induction occurred between 1 and 5 minutes after the injection (3 ± 1 minutes). The inductions were observed to be calm and smooth with no side effects. Vomiting was not observed in any of the lions. Five animals remained sleeping in sternal recumbency and never attained lateral recumbency but were immobilized; 15 lions went to lateral recumbency. The induction time ranged from 4-10 minutes (7 ± x minutes). There was no association between the variations in the BAM dose and the range of induction times recorded.

Immobilization was stable and no sudden arousals were observed. Good muscle relaxation was evident in all cases. Low jaw muscle tone disappeared within 10-15 minutes after injection, except for one female lion whose low jaw muscle tone was still present 22 minutes after administration. Capillary refill times were less than 2 seconds and mucous membrane colour was normal in all animals. Palpebral reflex disappeared at the 15^{th} minute of procedure and reappeared at the 45^{th}-50^{th} minute in the case of 12 animals, and at the 60^{th} minute – in the case of 8 animals. In two

219 animals, a weak palpebral reflex was registered during the entire period of
220 immobilization. Four lions exhibited spontaneous limb twitches during the first 20
221 minute of immobilization. None of the lions showed reaction to intubation,
222 extubation, or other painful procedures (e.g. blood collection). No apnoea was
223 observed in any of the lion. One hour after the beginning of the procedure, during
224 weighing, weak head and limb movements were observed (n = 14). The duration of
225 immobilization of one lion without additional doses was a little over one hour.

226 Table 1 presents the mean ± SD and range of the main monitoring variables
227 and Fig. 1 presents the measured physiological parameters during chemical restraint.
228 Mean blood pH level ($p < 0.001$) and lactate levels ($p < 0.001$) steadily declined in all
229 animals (Fig. 1). Administered dose (low: 0.52−0.56 mL 100 kg^{-1}; n = 6, medium:
230 0.59−0.64 mL 100 kg^{-1}; n = 8 and high: 0.66−0.83 mL 100 kg^{-1}; n = 6) did not
231 influenced any physiological parameters tested.

232 There was a significant difference in recovery time after administration of
233 naltrexone and yohimbine [22 ± 7 (7−34) minutes; n = 13] *versus* naltrexone and
234 atipamezole [9 ± 1 (8−1) minutes; n = 7] ($p < 0.001$) .

235

236 **Discussion**

237 The present study indicates that BAM (butorphanol-azaperone-medetomidine) is an
238 efficient immobilization protocol for lions. The results show that induction time is
239 similar to the butorphanol-medetomidine-midazolam combination (Wenger et al.
240 2010), slightly longer than the tiletamine-zolazepam-medetomidine combination
241 (Fahlman et al. 2005), but considerably shorter than the ketamine-xylazine
242 combination (Stander & Morkel 1991). The therapeutic dose rate of BAM solution is
243 wide and assures a high degree of reliability when using BAM under field conditions.

Based on the present study, the recommended dose of BAM for healthy lions is 0.6 mL 100 kg^{-1}. According to body weight, we recommend a total dose of 0.7−0.8 mL BAM for immobilization of an adult female or sub-adult male lion and a total dose of 1.0–1.2 mL for an adult male lion. The total volume of drug is lower than the total volume of other combinations (Fahlman et al. 2005; Stander & Morkel 1991) which allows for to the use of BAM with all types of remote delivery systems.

Few studies report physiological parameters in detail in immobilized lions. The physiological parameters recorded during the present study were stable and the cardiovascular parameters were within acceptable limits. A slight but stable bradycardia (defined as <50 beats $minute^{-1}$) was observed in all lions immobilized with BAM (Table 1). The HR was slightly lower than reported in other studies using tiletamine-zolazepam-medetomidine (Fahlman et al. 2005; Jacquier et al. 2006), butophanol-medetomidine-midazolam (Wenger et al. 2010) or ketamine and xylazine (Larsson et al. 2008). The HR was very similar to those in black bears immobilized with BAM (Wolf et al. 2008). The impact of dosage on HR and blood pressure does not seem to depend on the sex, body weight or the age of the animals. It can therefore be assumed that the bradycardia observed was because of the specific effect of medetomidine on peripheral α2-adrenoreceptors resulting in an increase of systemic vascular resistant (Sinclair 2003). Rectal temperature initially increased in 14 lions, and this is similar to observations in other studies using alpha-2-adrenoreceptor agonists (Fahlman et al. 2005; Jaquier et al. 2006; Wenger et al. 2010). Hyperthermia may have been caused by high ambient temperatures (19 – 38 °C) and interference with normal thermoregulatory mechanisms by alpha-2-adrenoreceptor agonists or opioids (Wenger et al. 2010). Arterial blood variables revealed the presence of mild metabolic acidosis during chemical restraint. Values for PaO_2, SaO_2, $PaCO_2$, pH and

lactate remained within reference ranges reported for domestic cats (King 2004). PaO_2 values were low and similar to values have been observed in lions immobilised with different drug combinations without additional oxygen supplementation (Fahlman et al. 2005; Wenger et al. 2010). Mean $PaCO_2$ in this study was 31.2 mmHg which is within the normal range reported for domestic cats, indicating adequate ventilation (King 2004). It was also similar to $PaCO_2$ values observed in lions immobilized with a butorphanol-medetomidine-midazolam combination (Wenger et al. 2010). The dose rate (low, medium or high) had no influence on heart rate, blood pressure or respiration parameters. This assures a high degree of reliability when practically applying the medication in field.

Using different reversal drugs clearly influences the recovery time and quality of recovery. The recovery time was significantly shorter when using naltrexone and atipamezole compared to when using naltrexone and yohimbine. Recovery with the naltrexone-atipamezole combination was smooth and occurred within 9 minutes. Animals showed signs of slight ataxia once standing during initial recovery. Recovery with the naltrexone-yohimbine combination was longer (22 ± 7 minutes) but still faster compared to the reported cases where a TZM combination was used in lions (mean time 33 minutes) (Fahlman et al. 2005). Thirteen lions, reversed with yohimbine were severely ataxic, which may be explained by the incomplete reversal of the medetomidine by yohimbine.

In conclusion, the BAM combination at the doses used in this study proved to be a reliable immobilization protocol for lions. Advantages of BAM include a small drug volume for darting, calm and smooth induction, long duration of immobilization and ability to reverse the effects of immobilization drugs with naltrexone and

atipamezole. Physiological parameters should be monitored throughout chemical restraint and additional oxygen supplementation may be necessary.

Authors' contributions Conception and design of the study, or acquisition of data, or interpretation of data: AS, VA, JPR, TO, DV, LL, SP; drafting of article or revising it critically: AS, VA, JPR, TO, DV, LL, SP; final approval of manuscript: AS, VA, JPR, TO, DV, LL, SP.

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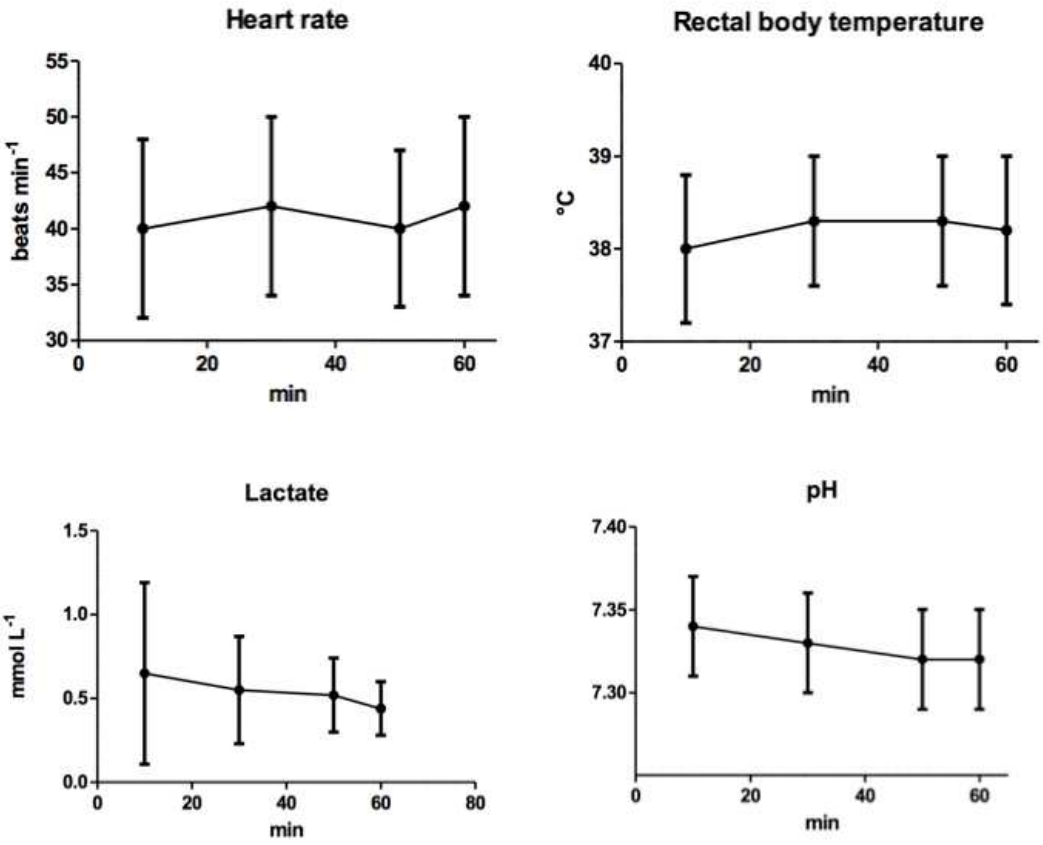
388 **Figure 1** The dynamics of heart rate, rectal body temperature, arterial blood pH and
389 lactate (mean ± SD) during immobilization of African lions (10-60 minutes after
390 darting) with a combination of butorphanol, azaperone, medetomidine (BAM).

391



392

Table 1 Physiologic variables and arterial blood gases in captive African lions darted with BAM (butorphanol-azaperone-medetomidine). Average values are presented for the periods 10−20, 25−35, 40−50 and 55−60 minutes after darting and for the entire immobilization (overall). Results are presented as mean ± standard deviation and (range)**.**

		Timepoint				
Variable	**Unit**	**10−20**	**25−35**	**40−50**	**55−60**	**Overall**
Heart rate	beats minute^{-1}	40 ± 8	42 ± 8	40 ± 7	42 ± 8	40 ± 8 (24-62)
Respiratory frequency	breaths minute^{-1}	15 ± 4	16 ± 4	16 ± 4	14 ± 3	15 ± 4 (6-30)
Rectal temperature	°C	38.0 ± 0.8	38.3 ± 0.7	38.3 ± 0.7	38.2 ± 0.8	38.2 ± 0.7 (36.6-39.5)
Systolic blood pressure	mmHg	175 ± 19	176 ± 23	170 ± 16	168 ± 14	170 ± 20 (118-236)
Diastolic blood pressure	mmHg	133 ± 11	130 ± 14	129 ± 12	129 ± 10	131 ± 16 (79-177)
Mean arterial pressure	mmHg	148 ± 14	143 ± 16	142 ± 11	137 ± 11	142 ± 16 (104-193)
SpO_2	%	86 ± 5	87 ± 5	89 ± 4	92 ± 4	88 ± 6 (72-100)
SaO_2	%	90 ± 4	92 ± 4	94 ± 4	94 ± 4	91 ± 5 (85-97)
PaO_2	mmHg	77 ± 4	76 ± 4	81 ± 5	82 ± 4	80 ± 4 (70-89)

$P_{E}´CO_2$	mmHg	39 ± 8	42 ± 7	41 ± 8	42 ± 6	41 ± 8 (18-59)
$PaCO_2$	mmHg	32.6 ± 5.7	30.1 ± 3.5	31.4 ± 3.5	32.1 ± 3.3	31.2 ± 3.4 (22.2-39.9)
pH		7.34 ± 0.03	7.33 ± 0.03	7.32 ± 0.03	7.32 ± 0.03	7.34 ± 0.03 (7.28-7.41)
Lactate	mmoL L^{-1}	0.65 ± 0.54	0.55 ± 0.32	0.52 ± 0.22	0.44 ± 0.16	0.54 ± 0.27 (0.30-2.64)

SpO_2, haemoglobin oxygen saturation measured by pulse oximetry; SaO_2, arterial haemoglobin saturation (calculated value, temperature corrected); PaO_2, partial pressure of arterial oxygen (measured value, temperature corrected); $P_{E}´CO_2$, end-tidal carbon dioxide measured by capnography; $PaCO_2$, partial pressure of arterial carbon dioxide (measured value, temperature corrected); pH corrected to rectal temperature.