

Supplementary Material



Supplementary Figure 1. The adapted face mask used to collect expired air in free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine, ketamine-medetomidine or ketamine-butorphanol-medetomidine. The mask was placed over the muzzle of the lion and made airtight by using a rectal glove gasket seal, which was secured onto the mask and face using tape. Expired air was redirected through air flow tubing to a gas mixing chamber and a respiratory flow head linked to a spirometer module of the PowerLab Exercise Physiology System (ADInstruments).

Supplementary Table 1 Measured and calculated variables from free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine (KM) or ketamine-butorphanol-medetomidine (KBM) (n = 12 per drug combination).

Variable	Measured	Calculated
<u>Digital thermometer</u>		
Rectal temperature	✓	
<u>Powerlab¹</u>		
Respiratory rate (fR)	✓	
Expired minute ventilation at standard body temperature and pressure (VE _{BTFS})	✓	
Oxygen consumption (VO ₂)		✓
Carbon dioxide production (VCO ₂)		✓
Respiratory exchange ratio (RER)		✓
<u>EPOC blood gas analyser²</u>		
pH	✓	
Partial pressure of arterial oxygen (PaO ₂)	✓	
Partial pressure of arterial carbon dioxide (PaCO ₂)	✓	
Base excess		✓
Bicarbonate (HCO ₃ ⁻)		✓
Barometric pressure (P _b)	✓	
Haemoglobin concentration ([Hb])		✓
Haemoglobin saturation of oxygen in arterial blood (SaO ₂)		✓
<u>Manual calculations</u>		
Expected minute ventilation (VE _{EXP})		✓
Tidal volume (VT)		✓
Expected tidal volume (VT _{EXP})		✓
Alveolar-arterial oxygen partial pressure gradient (P(A-a)O ₂)		✓
Alveolar vapor pressure of saturated air at specific body temperature (P _{H₂O})		✓
Expected partial pressure of arterial oxygen (PaO _{2EXP})		✓

1, Calculated variables obtained from the Powerlab system were calculated by LabChart 7 software (ADIstruments)

2, Calculated variables obtained from the EPOC blood gas analyser were calculated using human-derived formulae

Supplementary Table 2 Ventilation at standard body temperature and pressure (VE_{BTPS}), respiratory rate and tidal volume values from free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine (KM) or ketamine-butorphanol-medetomidine (KBM) (n = 12 per drug combination).

Sampling time	Distribution	VE_{BTPS} (L/min)			Respiratory rate (breaths/min)			Tidal volume (mL/breath)		
		TZM	KM	KBM	TZM	KM	KBM	TZM	KM	KBM
	Expected mean \pm SD	28.7 \pm 3.2	26.6 \pm 4.5	30.8 \pm 5.6				1900 \pm 500	1800 \pm 600	2100 \pm 500
0	Mean	27.2	26.1	23.2	16	17	15	1800	1580	1600
	SD	9.5	14.3	10.8	3	7	2	710	470	730
10	Mean	29.5	27.6	25.2	16	17	15	1950	1560	1740
	SD	15.9	18.4	12.2	3	8	3	1120	490	810
20	Mean	30.9	27.4	25.3	15	18	15	2090	1520	1720
	SD	18.3	18.4	12.8	3	9	2	1360	450	800
30	Mean	35.1 [*]	28.4 [†]	26.7 [#]	15	17	15	2380 [*]	1640 [†]	1820 [#]
	SD	25.4	18.4	14.2	3	7	3	1930	500	880

* Significantly different from measurement at T_0 in TZM group

† Significantly different from measurement at T_0 in KM group

Significantly different from measurement at T_0 in KBM group

^a Significantly different from animals immobilised with KM at specific sampling point

^b Significantly different from animals immobilised with TZM at specific sampling point

^c Significantly different from animals immobilised with KBM at specific sampling point

Supplementary Table 3 Oxygen consumption (VO₂), carbon dioxide production (VCO₂) and respiratory exchange ratio (RER) values from free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine (KM) or ketamine-butorphanol-medetomidine (KBM) (n = 12 per drug combination).

Sampling time	Distribution	VO ₂ (L/min)			VCO ₂ (L/min)			RER		
		TZM	KM	KBM	TZM	KM	KBM	TZM	KM	KBM
0	Mean	0.6	0.5	0.5	0.5 ^c	0.4	0.4 ^b	0.9	0.9	0.9
	SD	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.1	0.1
10	Mean	0.6	0.5	0.5	0.5 ^c	0.5	0.4 ^b	0.9	0.9	0.9
	SD	0.3	0.2	0.2	0.3	0.2	0.2	0.0	0.1	0.1
20	Mean	0.6	0.5	0.5	0.6 ^c	0.5	0.4 ^b	0.9	1.0	0.9
	SD	0.4	0.1	0.2	0.3	0.2	0.2	0.0	0.1	0.1
30	Mean	0.7	0.5	0.5	0.7 ^{*c}	0.5 [*]	0.5 ^{#b}	0.9	1.0	0.9
	SD	0.5	0.1	0.3	0.5	0.1	0.2	0.1	0.1	0.1

* Significantly different from measurement at T₀ in TZM group

† Significantly different from measurement at T₀ in KM group

Significantly different from measurement at T₀ in KBM group

^a Significantly different from animals immobilised with KM at specific sampling point

^b Significantly different from animals immobilised with TZM at specific sampling point

^c Significantly different from animals immobilised with KBM at specific sampling point

Supplementary Table 4 Partial pressure of arterial oxygen (PaO₂) and carbon dioxide (PaCO₂) in arterial blood, partial pressure of alveolar oxygen (PAO₂) and alveolar-arterial (P(A-a)O₂) gradient from free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine (KM) or ketamine-butorphanol-medetomidine (KBM) (n = 12 per drug combination; *n = 11).

Sampling time	Distribution	PaO ₂ (mmHg)			PaCO ₂ (mmHg)			PAO ₂ (mmHg)			(P(A-a)O ₂) Gradient		
		TZM	KM	KBM	TZM	KM	KBM	TZM	KM	KBM	TZM	KM	KBM
0	Mean	78.5 ^c	76.7	74.0 ^b	32.6 ^c	32.6 ^c	34.5 ^{ab}	106.9	109.6	103.7	27.8	32.7	29.6
	SD	4.7	6.3	7.8	2.2	3.8	4.2	2.8	9.1	6.4	2.8	11.7	4.5
10	Mean	82.7 [*]	80.1 [†]	78.9 [#]	32.3	32.7	34.3	108.4	108.8	104.8	24.7 [*]	28.8 [†]	25.9 [#]
	SD	3.7	7.2	8.6	2.8	4.1	4.4	3.3	6.1	6.9	3.9	9.3	4.2
20	Mean	86.2 [*]	82.3 [†]	79.7 [#]	32.7	32.5	36.2	109.1	110.0	103.7	22.9 [*]	27.8 [†]	24.0 [#]
	SD	4.1	6.4	8.5	3.2	3.4	4.0	3.9	6.2	6.1	3.7	11.2	5.0
30	Mean	86.1 ^{*c}	84.7 [†]	82.4 ^{#b}	33.3 ^c	32.0 ^c	34.9 ^{ab}	109.2	114.3	105.7	21.8 [*]	26.9 [†]	23.5 [#]
	SD	2.0	6.7	8.0	2.0	4.7	5.2	3.7	11.5	6.4	4.6	10.4	4.8

* Significantly different from measurement at T₀ in TZM group

† Significantly different from measurement at T₀ in KM group

Significantly different from measurement at T₀ in KBM group

^a Significantly different from animals immobilised with KM at specific sampling point

^b Significantly different from animals immobilised with TZM at specific sampling point

^c Significantly different from animals immobilised with KBM at specific sampling point

Supplementary Table 5 pH, bicarbonate (HCO₃⁻) and base excess of arterial blood from free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine (KM) or ketamine-butorphanol-medetomidine (KBM) (n = 12 per drug combination; *n = 11).

Sampling time	Distribution	pH			HCO ₃ ⁻ (mmol/L)			Base excess (mmol/L)		
		TZM	KM	KBM	TZM	KM	KBM	TZM	KM	KBM
0	Mean	7.46 ^c	7.45 ^c	7.41 ^{ab}	23.3	22.6	22.0	0.0	-0.8	-2.1
	SD	0.03	0.03	0.03	2.0	3.0	3.3	2.2	2.8	3.1
10	Mean	7.45 ^c	7.45 ^c	7.41 ^{ab}	22.6	22.8	21.9	-0.8	-0.6	-2.2
	SD	0.03	0.04	0.02	2.4	3.0	3.5	2.5	2.9	3.3
20	Mean	7.45 ^c	7.45 ^c	7.40 ^{ab}	22.7	22.5	22.6	-0.8	-0.9	-1.8
	SD	0.03	0.03	0.02	2.1	2.2	2.9	1.9	2.1	2.6
30	Mean	7.44 ^c	7.45 ^c	7.41 ^{ab}	22.6	22.2	22.0	-1.1	-1.2	-2.3
	SD	0.02	0.03	0.02	1.8	2.8	3.4	1.8	2.4	3.1

^a Significantly different from animals immobilised with KM at specific sampling point

^b Significantly different from animals immobilised with TZM at specific sampling point

^c Significantly different from animals immobilised with KBM at specific sampling point

Supplementary Table 6 Body temperatures from free-living African lions (*Panthera leo*) immobilised with tiletamine-zolazepam-medetomidine (TZM), ketamine-medetomidine (KM) or ketamine-butorphanol-medetomidine (KBM) (n = 12 per drug combination).

Sampling time	Distribution	Body temperature (°C)		
		TZM	KM	KBM
0	Mean	39.5	39.7	39.5
	SD	0.5	1.2	0.5
10	Mean	39.5	39.7	39.4
	SD	0.5	1.3	0.6
20	Mean	39.3*	39.5 [†]	39.3 [#]
	SD	0.5	1.3	0.6
30	Mean	39.2*	39.5 [†]	39.0 [#]
	SD	0.5	1.2	0.6

* Measurement significantly different from measurement at T₀ in TZM group

[†] Measurement significantly different from measurement at T₀ in KM group

[#] Measurement significantly different from measurement at T₀ in KBM group

^a Significantly different from animals immobilised with KM at specific sampling point

^b Significantly different from animals immobilised with TZM at specific sampling point

^c Significantly different from animals immobilised with KBM at specific sampling point