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Blood oxygen depletion during rest-associated apneas of northern elephant seals (*Mirounga angustirostris*)

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Summary

Blood gases (P_{O_2}, P_{CO_2}, pH), oxygen content, hematocrit and hemoglobin concentration were measured during rest-associated apneas of nine juvenile northern elephant seals. In conjunction with blood volume determinations, these data were used to determine total blood oxygen stores, the rate and magnitude of blood O_2 depletion, the contribution of the blood O_2 store to apneic metabolic rate, and the degree of hypoxemia that occurs during these breath-holds. Mean body mass was 66±9.7 kg (± s.d.); blood volume was 196±20 ml kg^{-1}; and hemoglobin concentration was 23.5±1.5 g dl^{-1}. Rest apneas ranged in duration from 3.1 to 10.9 min. Arterial P_{O_2} declined exponentially during apnea, ranging between a maximum of 108 mmHg and a minimum of 18 mmHg after a 9.1 min breath-hold. Venous P_{O_2} values were indistinguishable from arterial values after the first minute of apnea; the lowest venous P_{O_2} recorded was 15 mmHg after a 7.8 min apnea. O_2 contents were also similar between the arterial and venous systems, declining linearly at rates of 2.3 and 2.0 ml O_2 dl^{-1} min^{-1}, respectively, from mean initial values of 27.2 and 26.0 ml O_2 dl^{-1}. These blood O_2 depletion rates are approximately twice the reported values during forced submersion and are consistent with maintenance of previously measured high cardiac outputs during rest-associated breath-holds. During a typical 7-min apnea, seals consumed, on average, 56% of the initial blood O_2 store of 52 ml O_2 kg^{-1}; this contributed 4.2 ml O_2 kg^{-1} min^{-1} to total body metabolic rate during the breath-hold. Extreme hypoxemic tolerance in these seals was demonstrated by arterial P_{O_2} values during late apnea that were less than human thresholds for shallow-water blackout. Despite such low P_{O_2}s, there was no evidence of significant anaerobic metabolism, as changes in blood pH were minimal and attributable to increased P_{CO_2}. These findings and the previously reported lack of lactate accumulation during these breath-holds are consistent with the maintenance of aerobic metabolism even at low oxygen tensions during rest-associated apneas. Such hypoxemic tolerance is necessary in order to allow dissociation of O_2 from hemoglobin and provide effective utilization of the blood O_2 store.

Key words: blood gases, oxygen, P_{O_2}, elephant seal, apnea.

Introduction

Elephant seals, renowned for their diving ability and extensive pelagic lifestyle, spend 2–4 months per year on land in order to breed or molt. During these terrestrial phases, they exhibit prolonged, natural breath-holds while asleep (Bartholomew, 1954; Blackwell and Le Boeuf, 1993; Kenny, 1979). In northern elephant seals (*Mirounga angustirostris*), such breath-holds can last as long as 25 min, with mean durations of 7–10 min in animals ranging from older weanlings to adults (Blackwell and Le Boeuf, 1993). These sleep apneas are considered to conserve energy and water during the on-shore fast (Ortiz et al., 1978), and it has been documented that the seals spend 60–70% of time during sleep in apnea (Blackwell and Le Boeuf, 1993). It is presumed that significant aerobic metabolism continues during these apneas because of (1) the lack of lactate accumulation (Castellini, 1994; Castellini et al., 1986) and (2) the maintenance of heart rate, cardiac output and presumed perfusion of splanchnic organs during sleep apnea (Ponganis et al., 2006). Continued aerobic metabolism during these prolonged breath-holds would be dependent on significant depletion of a large blood O_2 store, a rapid blood O_2 depletion rate (relative to forced submersion) and extreme hypoxemic tolerance. The latter is essential in order to achieve a low enough oxygen partial pressure (P_{O_2}) to allow depletion of the blood O_2 store.

We investigated the change in blood gases and the depletion of blood O_2 during sleep apneas of young elephant seals in order to determine (1) the magnitude of the pre-apneic blood O_2 store, (2) the percentage of this store consumed during a breath-hold, and its contribution to total metabolic rate during the apnea, (3) the rate of apneic blood O_2 utilization and (4) the degree of hypoxemia that occurs during apnea. We hypothesized that the blood O_2 store would be significantly depleted during rest apneas, the blood O_2 depletion rate would be greater than that during forced submersion, and the hypoxemic tolerance of the

seals would allow them to tolerate P_{O_2} values less than 25–30 mmHg [a threshold at which humans lose consciousness (Ferretti et al., 1991; Ferrigno and Lundgren, 2003)], thereby enabling them to extract more oxygen from blood.

Materials and methods

Animals

Juvenile northern elephant seals (*Mirounga angustirostris* Gill), two females and seven males, aged 5–8 months, 51–86 kg, were obtained from the rehabilitation program of Sea World of San Diego and housed at Scripps Institution of Oceanography for 1–2 months each summer from 2001 to 2003. Animals were maintained on a diet of frozen smelt (10% of body mass per day) and released at sea following completion of each season's experiments. (Federal Marine Mammal Permit # 732-1487; UCSD Animal Subjects Protocol # S-00092.)

Catheterizations and instrumentation

Catheterization of the brachial or femoral artery, extradural vein (EDV), hepatic sinus (HS), and thoracic vena cava (TVC)–heart–pulmonary artery (PA) were accomplished as previously described (Ponganis et al., 2006). A given seal was instrumented either with arterial and EDV catheters, or with a suite of venous/cardiac catheters. Catheters were inserted percutaneously into seals under general 1–2% isoflurane–O_2 anesthesia after mask induction and intubation. Catheter position was confirmed by characteristic pressure wave forms (Hewlett Packard 78302A/78205D pressure monitor; Palo Alto, CA, USA) and fluoroscopy before being secured with neoprene, Velcro™ and Loctite 401 glue (Loctite Corporation, Rocky Hill, CT, USA).

Transthoracic surface electrocardiogram (ECG) electrodes were affixed with rubber and Loctite glue and attached to an ECG/impedance monitor (RespI/ECG; UFI, Morro Bay, CA, USA). After extubation, seals were placed unrestrained in an open-framework PVC cage, which allowed easy access to catheters and ECG cables under a draped blind. The animals were left undisturbed, usually with hose water running over the bottom of the cage. The ECG and thoracic impedance (transduced from the ECG electrodes) were recorded to computer continuously at 100 Hz and 25 Hz sampling rates, respectively, with a BIOPAC Systems Model MP100 analog-to-digital interface, using AcqKnowledge software (BIOPAC Systems, Goleta, CA, USA). Blood sampling times were marked in the computer record.

The seal was allowed to recover from anesthesia for at least 6 h before blood samples were drawn at the resumption of prolonged (>3 min), spontaneous apneas. Blood was drawn intermittently during eupneas and serially during apneas. At the end of the study, catheters and ECG electrodes were removed after 0.5 mg kg^{-1} ketamine intravenous sedation. Prophylactic cephalexin was administered intravenously (1 g every 6 h) while the seals were instrumented, and then orally (250 mg, three times per day) for three days afterwards.

Blood sampling, [hemoglobin], hematocrit and blood volume assays

Duplicate 1-ml blood samples were drawn into pre-heparinized blood gas syringes (Marquest™ Gaslyte®; Marquest Medical Products, Englewood, CO, USA) with extra 0.05 ml heparin (10 000 U ml^{-1}) in the hub. One sample was used for blood gas analysis; the duplicate for hematocrit (Hct) and hemoglobin concentration ([Hb]) determinations. Blood analyses for P_{O_2}, P_{CO_2}, pH and oxygen saturation (sO_2%) were performed at 37°C with an i-STAT Portable Clinical Blood Analyzer (i-STAT Corporation, East Windsor, NJ, USA) using G3+ cartridges (Abbott Laboratories, Abbott Park, IL, USA). [Hb] was determined by a cyanmethemoglobin colorimetric assay, using either the Sigma #525 Total Hemoglobin Test Kit (Sigma Diagnostics, St Louis, MO, USA) or the Teco Diagnostics (Anaheim, CA, USA) total hemoglobin kit. Hematocrit was determined by microcentrifugation. Blood oxygen contents presented in this paper were calculated by assuming 1.34 ml O_2 g^{-1} Hb and multiplying by the percent oxygen saturation from the i-STAT analyzer and the [Hb] result from the assay (Qvist et al., 1986). Accuracy of this calculation was assessed in one experiment in which blood oxygen content measurements (Tucker, 1967) were made with the use of a borrowed Tucker chamber and oxygen electrode (Strathkelvin Instruments, Motherwell, UK: Tucker chamber, model 1302 electrode, and 781 meter).

Plasma volume (V_P) was determined using the Evans blue dye dilution technique (Castellini et al., 1987) either during anesthesia or at the end of an experiment (4 ml of a 10 mg ml^{-1} dye stock solution were injected). Samples were drawn at 5, 10, 15, 20 and 30 min after injection. Absorbances were read at 624 nm and compared to a standard curve made in the individual seal's blank plasma (collected prior to injection of the dye). To calculate blood volume (V_B), the highest Hct recorded during an apnea with a given seal was used in the formula $V_B=V_P/(1–Hct)$ (Ponganis et al., 1993).

Data analysis

Data from eupneic and apneic periods were sorted according to the respiratory impedance trace on the continuous computer record. An apnea was defined as any breath-hold longer than 3 min duration. In order to conduct analyses over a wide range of apneic durations, data from a given blood sampling site were pooled from all animals and analyzed together.

Venous samples were categorized into three compartments for comparison: pulmonary artery (PA), central venous (CV: right atrium (RA), thoracic vena cava, hepatic sinus) or extradural vein (EDV).

Data were graphed and analyzed with Excel (Microsoft), Origin (Microcal Software, Northampton, MA, USA) and SPSS (Chicago, IL, USA) software. All means are ± 1 s.d. Statistical significance was assumed at $P<0.05$. Because the output of the blood gas analyzer was in mmHg, those units will be presented in tables and curve-fitting equations throughout this paper. In the graphs, however, dual axes for both mmHg and kPa will be shown (1 mmHg=0.133 kPa).

Results

Experimental summary

Blood samples were collected from nine seals (mean body mass, 66±9.7 kg) during 11 experiments (Tables 1, 2). Apneas appeared to occur always during sleep and ranged in duration from 3.1 to 10.9 min, with individual mean values of

Table 1. *Characteristics of elephant seals used in experiments*

Seal I.D.	Body mass (kg)	Mass-specific plasma volume (ml kg^{-1}) (Hct)*	Mean [Hb] blood volume (ml kg^{-1})	Range of during apnea (g dl^{-1}) (*N*)	Mean apnea durations (min) (*N*)	apnea duration (min) (± s.d.)
B	75	70 (62)	184	22.8±1.8 (15)	4.8–10.2 (12)	6.6±1.5
I	86	74 (69)	239	24.0±0.9 (11)	3.9–10.8 (7)	7.2±2.4
K	67	93 (50)	186	22.7±1.2 (33)	3.3–7.7 (17)	5.3±1.2
Mi	51	–	–	21.9±2.1 (30)	3.1–7.1 (12)	5.8 ±1.2
Mo	57	88 (51)	180	21.4±1.1 (12)	4.0–6.3 (7)	5.4±0.7
R	59	–	–	22.3±1.4 (17)	3.5–5.7 (14)	4.8±0.6
S	73	85 (56)	193	24.7±1.6 (20)	3.2–7.1 (12)	5.1±1.2
W1	58	–	–	23.2±2.0 (25)	3.5–8.0 (14)	7.0±1.1
W2	66	76 (61)	195	24.4±0.8 (27)	4.0–7.6 (9)	6.0±1.5
Z1	65	–	–	26.5±1.7 (31)	3.7–10.9 (13)	7.3±2.3
Z2	65	78 (60)	195	24.8±2.0 (23)	5.0–9.2 (14)	6.7±1.4
Mean ± s.d.	66±9.7	81±8.3 (58±6.7)	196±19.8	23.5±1.5		

*Hct value used for calculation of blood volume was the highest hematocrit (Hct) observed during an experiment.

4.8–7.3 min (Table 1). Mean V_B was 196±20 ml kg^{-1} (*N*=7) (Table 1), and mean [Hb] during apnea was 23.5±1.5 g dl^{-1} (*N*=11). The numbers of samples collected per sampling site per animal are presented in Table 2.

Calculated vs measured O_2 content

A linear regression analysis was performed on calculated *vs* measured O_2 content values of 24 blood samples from one seal (W1). The resulting equation was: y=1.003x+1.1 (where y is calculated O_2 content and x is measured O_2 content); r^2=0.90; P<0.0001.

Blood gases, O_2 content, pH and [Hb] during eupnea

During the eupneic periods between rest-associated apneas, the blood gas values were widely variable; ranges are presented in Table 3. Variability was evident both within and between individual seals. Ranges of venous P_{O_2} and P_{CO_2} were quite similar among the three venous compartments (PA, CV and EDV). Overall, the venous P_{O_2} varied between 34 and 71 mmHg, while the arterial spanned 40 to 108 mmHg; venous P_{CO_2} ranged from 45 to 64 mmHg and arterial from 35 to 56 mmHg. Ranges of pH, [Hb] and O_2 content were similar for

Table 2. *Summary of arterial and venous sampling sites in all seals*

Seal I.D.	Artery	Venous sites				
		PA	RA	TVC	HS	EDV
B	15					
I	11					
K	16	9	8			
Mi	16	14				
Mo	12					
R	17					
S	20					
W1	10	6	10			
W2	14	1	12			
Z1	22	9				
Z2	14	9				

For each sampling site, the number of samples drawn during apneas is given. Total number of arterial samples = 82; total number of venous samples = 163

PA, pulmonary artery; RA, right atrium; TVC, thoracic vena cava; HS, hepatic sinus; EDV, extradural vein.

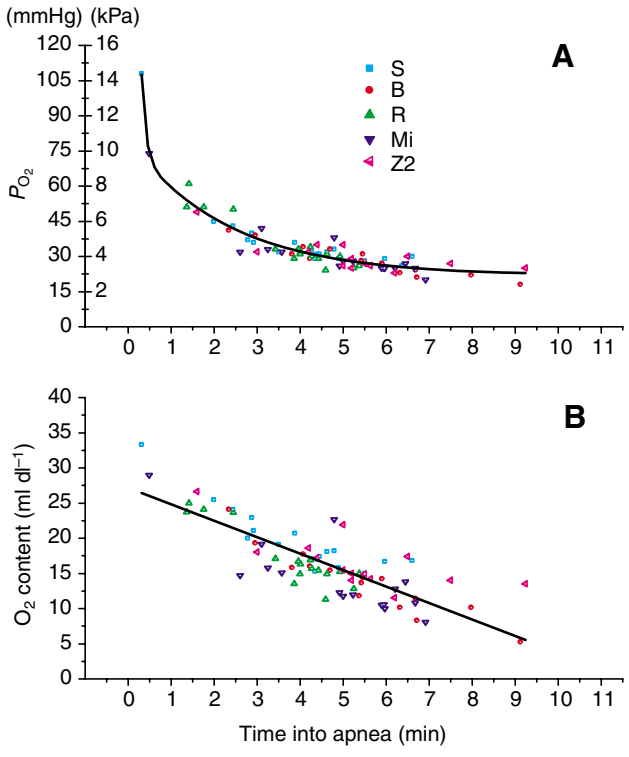

Fig. 1. Changes in arterial P_{O_2} (A) and O_2 content (B) as a function of time into apnea. Points in A and B are individual blood samples from five seals (see Table 2 for labels of individual seals). (A) Equation for exponential rate of P_{O_2} decline: y=58e$^{(-x/2.32)}$+765e$^{(-x/0.10)}$+21.8 (r^2=0.93, N=82). (B) Equation for linear depletion of O_2 content: y=–2.34x+27.2 (r^2=0.68, N=82, P<0.0001).

Table 3. *Range of eupneic blood values from all experiments*

Sampling site	P_{O_2} (mmHg)	O_2 content (ml dl^{-1})	P_{CO_2} (mmHg)	[Hb] pH	(g dl^{-1})	*N*
Arterial	40–108	19.6–35.0	35–56	7.29–7.42	19.9–27.7	34
B	83–106	29.2–34.3	35–44	7.30–7.38	22.7–27.7	7
Mi	40–82	19.6–31.7	49–56	7.34–7.38	20.3–24.3	7
R	44–108	21.5–28.8	40–50	7.32–7.42	19.9–21.7	8
S	50–97	29.4–35.0	40–47	7.29–7.36	23.5–27.0	7
Z2	84–105	30.1–34.7	44–51	7.33–7.38	22.9–27.0	5
Venous						
PA	34–57	20.0–28.8	47–59	7.31–7.38	20.0–24.8	8
K	42–51	20.0–24.4	47–59	7.31–7.38	20.0–22.3	5
W2	34–57	20.6–28.8	49–49	7.31–7.37	24.3–24.8	3
CV	37–68	20.0–32.9	45–61	7.29–7.36	20.0–28.8	16
K	40–55	20.0–24.4	50–55	7.31–7.36	20.0–21.5	4
W1	61–68	26.5–30.0	46–46	7.34–7.36	21.8–24.2	2
Z1	37–66	23.3–32.9	45–61	7.29–7.36	23.9–28.8	10
EDV	34–71	18.6–32.6	47–64	7.27–7.36	20.0–27.0	29
I	39–65	21.3–28.9	47–51	7.29–7.31	23.0–25.1	7
Mi	37–51	18.6–28.3	53–59	7.30–7.36	20.7–25.6	6
Mo	41–71	20.5–25.8	50–60	7.30–7.34	20.0–21.9	7
W2	34–59	21.0–29.2	48–50	7.35–7.35	23.7–24.7	3
Z1	36–69	20.8–32.6	48–64	7.27–7.34	23.9–27.0	6

both arterial and venous blood during eupnea. Mean eupneic Hct of venous and arterial blood among seven seals was 53±5.0% (*N*=60).

P_{O_2} and O_2 content during apnea

Arterial P_{O_2}s declined exponentially during apnea from initial values as high as 108 mmHg to a minimum of 18 mmHg after 9.1 min of apnea (Fig. 1A). Arterial O_2 contents (Fig. 1B), which averaged 27.2 ml dl^{-1} at the start of apnea, declined linearly to an average of 6.0 ml dl^{-1} by 9 min. This represented a depletion rate of 2.3 ml O_2 dl^{-1} min^{-1}. The lowest O_2 content was 5.2 ml dl^{-1} after 9.1 min.

Venous P_{O_2} values in the EDV, CV and PA each declined exponentially, while O_2 contents decreased linearly in these three compartments (Fig. 2). The data from the different venous compartments were also pooled to construct curve fits for a combined venous response (black lines in Fig. 2). On average, the initial oxygen tension in the venous system was 59 mmHg and decreased to 21 mmHg after 9 min of apnea (Fig. 2A). The lowest P_{O_2} recorded was 15 mmHg after a 7.8 min apnea. Venous O_2 content at the onset of apnea was 26.0 ml dl^{-1} on average and decreased linearly, at a rate of 2.0 ml dl^{-1} min^{-1}, to 7.8 ml dl^{-1} by 9 min (Fig. 2B). The lowest venous O_2 content recorded was 4.8 ml dl^{-1} after a 7.1 min apnea.

Analysis of simultaneous, paired PA and EDV apneic samples from one seal (W2) revealed that only P_{O_2} and O_2 content were significantly different, with the EDV values being consistently higher than the PA values (mean P_{O_2} difference,

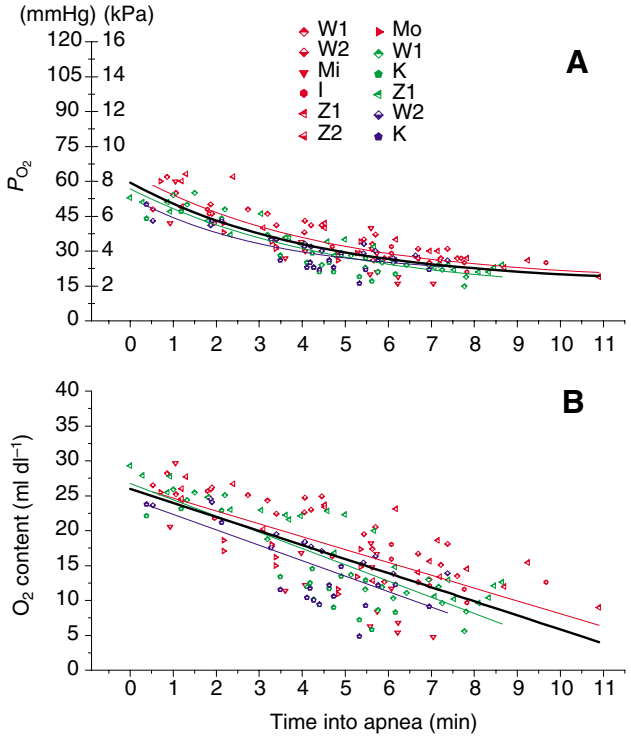

Fig. 2. Changes in venous P_{O_2} (A) and O_2 content (B) with time into apnea. Individual data points (see Table 2 for labels of seals) from each of the three venous compartments are indicated by color: extradural vein (EDV; red), central veins (CV; green) and pulmonary artery (PA; blue); curve fits indicating compartmental trends are shown in the same respective colors. (A) Equation, from all data pooled (black line), for exponential rate of P_{O_2} decline: $y=43.7e^{(-x/4.30)}+15.8$ (r^2=0.71, *N*=163). (B) Equation from pooled data (black line) for linear depletion rate of O_2 content: $y=-2.01x+26.0$ (r^2=0.54, *P*<0.0001, *N*=163).

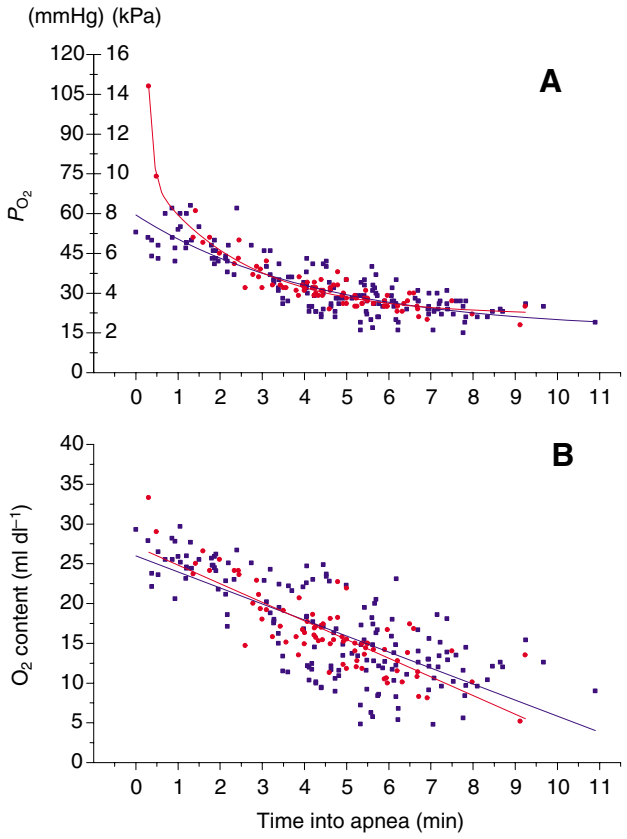

Fig. 3. Comparison of arterial (red) and venous (blue) blood in terms of P_{O_2} (A) and O_2 content (B). The venous system is highly saturated; P_{O_2} and O_2 content in both the arterial and venous blood become indistinguishable soon into apnea. (Regression equations are the same as in Figs 1 and 2.)

7±2.87 mmHg; t=7.661, P<0.0001, N=11; mean O_2 content difference, 4.1±1.96 ml O_2 dl^{-1}; t=6.667, P<0.0001, N=10). Paired PA–HS apneic samples in seal K revealed a significant difference only in O_2 content, with HS having slightly higher content than PA (mean O_2 content difference, 1.10±1.06 ml O_2 dl^{-1}; t=2.730, P=0.034). No significant differences were found in any blood parameter for paired PA–RA apneic samples from seal K.

Fig. 3A overlays the arterial and venous P_{O_2} results to illustrate that the oxygen tensions were similar in both systems after the first minute of apnea. O_2 contents were also similar in the arterial and venous systems (Fig. 3B).

P_{CO_2}, pH, [Hb] and Hct during apnea

Changes in arterial and venous P_{CO_2}, pH, [Hb] and Hct during apnea are shown in Fig. 4. During apnea, mean P_{CO_2} rose gradually from initial values of 49 (arterial) and 52 (venous) mmHg to 63 mmHg by 9 min. pH was identical in both systems and, on average, decreased less than 0.05 units during a 9 min apnea. [Hb] and Hct were scattered but showed a slowly increasing trend with apnea duration. Arterial and venous [Hb] in all animals ranged between 19 and 28 g dl^{-1}, while Hct ranged between 47 and 67%.

End-of-apnea blood values

End-of-apnea values of P_{O_2}, O_2 content, P_{CO_2} and pH from arterial blood samples drawn within the last minute of apnea were plotted against apnea duration (Fig. 5). End values of P_{O_2} and O_2 content continued to decrease as apnea duration increased. P_{CO_2} increased gradually with increasing apnea duration, while pH remained essentially unchanged. There was no apparent critical value of any blood parameter that dictated the end of apnea or limited the duration of these rest-associated apneas.

Discussion

Experimental protocol

The elephant seals in this study had previously beached during their first trip to sea but had been successfully rehabilitated and were considered in good health, as evidenced by veterinary examinations, good appetite and weight gain, and ready condition for release to sea. Although the average mass of these seals was less than that of 8-month-old juveniles following their first trip to sea (Thorson and Le Boeuf, 1994), seals in this size range do successfully complete these trips (Hindell et al., 1999; Le Boeuf et al., 1994; McConnell et al., 2002; Thorson and Le Boeuf, 1994). In addition, their Hct, [Hb], V_B and heart rate responses were in the same ranges as those of healthy, young elephant seals both in the wild and in captivity (Andrews et al., 1997; Castellini et al., 1994a; Castellini et al., 1994b; Falabella et al., 1999; Ponganis et al., 2006; Simpson et al., 1970; Thorson and Le Boeuf, 1994). Therefore, as already reviewed by Ponganis et al. (Ponganis et al., 2006), we do not think that the data collected in this study were compromised by the rehabilitated status of these seals.

It should be noted that mass-specific V_P and V_B increase during the first year of life in seals. These increases are probably secondary to hematopoietic processes and to changes in body mass/composition. Plasma volume and V_B are 45 ml kg^{-1} and 110 ml kg^{-1}, respectively, in northern elephant seal pups (Castellini and Castellini, 1993; Castellini et al., 1987), 74 ml kg^{-1} (estimated from mean Hct and V_B data) and 175 ml kg^{-1}, respectively, in 90 kg, 8-month-old juvenile elephant seals on return from their first trip to sea (Thorson and Le Boeuf, 1994), and 63–98 ml kg^{-1} (estimated from individual Hct and V_B data) and 175–256 ml kg^{-1} in captive 6-month to 3-year-old juvenile elephant seals (Simpson et al., 1970). The values of the seals in this study are closest to those of the young juveniles in the latter two studies.

Blood sampling and data collection during an experiment began after the seal resumed spontaneous, long (>3 min) apneas, and at least 6 h post-anesthesia. By this time, the heart rate had also resumed normal sinus arrhythmic patterns, equivalent to those recorded from seals more than 24 h post-anesthesia (P.J.P. and T.K.S., unpublished data) (Castellini et al., 1994b). For these reasons, and because of the fast wash-out of isoflurane in pinnipeds (Gales, 1998), it was concluded that there was no residual effect of anesthesia on the data.

In this study, we assumed that a seal was asleep during breath-holds if we observed closed eyes, occasional facial twitching and lack of body movement. These signs are indicative of sleep apnea, as documented by electroencephalography (EEG) (Castellini et al., 1994a).

However, because we had no direct EEG evidence of sleep state in our seals, we have referred to these breath-holds as 'rest-associated' apneas.

In order to have sufficient data to profile a given parameter over a range of apneic durations, it was necessary to pool the data from different breath-holds and from different animals for analysis. This was due to (1) catheter malfunctions, (2) the amount of time required to clear dead space, draw samples and flush lines, and (3) the inability to predict when a given apnea would start and end. A univariate analysis of covariance test (SPSS) of O_2 contents revealed that there were no significant differences among either venous or arterial samples when the interaction between individual seal and time into apnea was tested (venous, F=1.615, P=0.101; arterial, F=2.058, P=0.096). Nevertheless, it is acknowledged, and should be noted by the reader, that this pooling approach is limited in that it does not allow evaluation of differences between individual seals or within apneas of different durations. However, given the

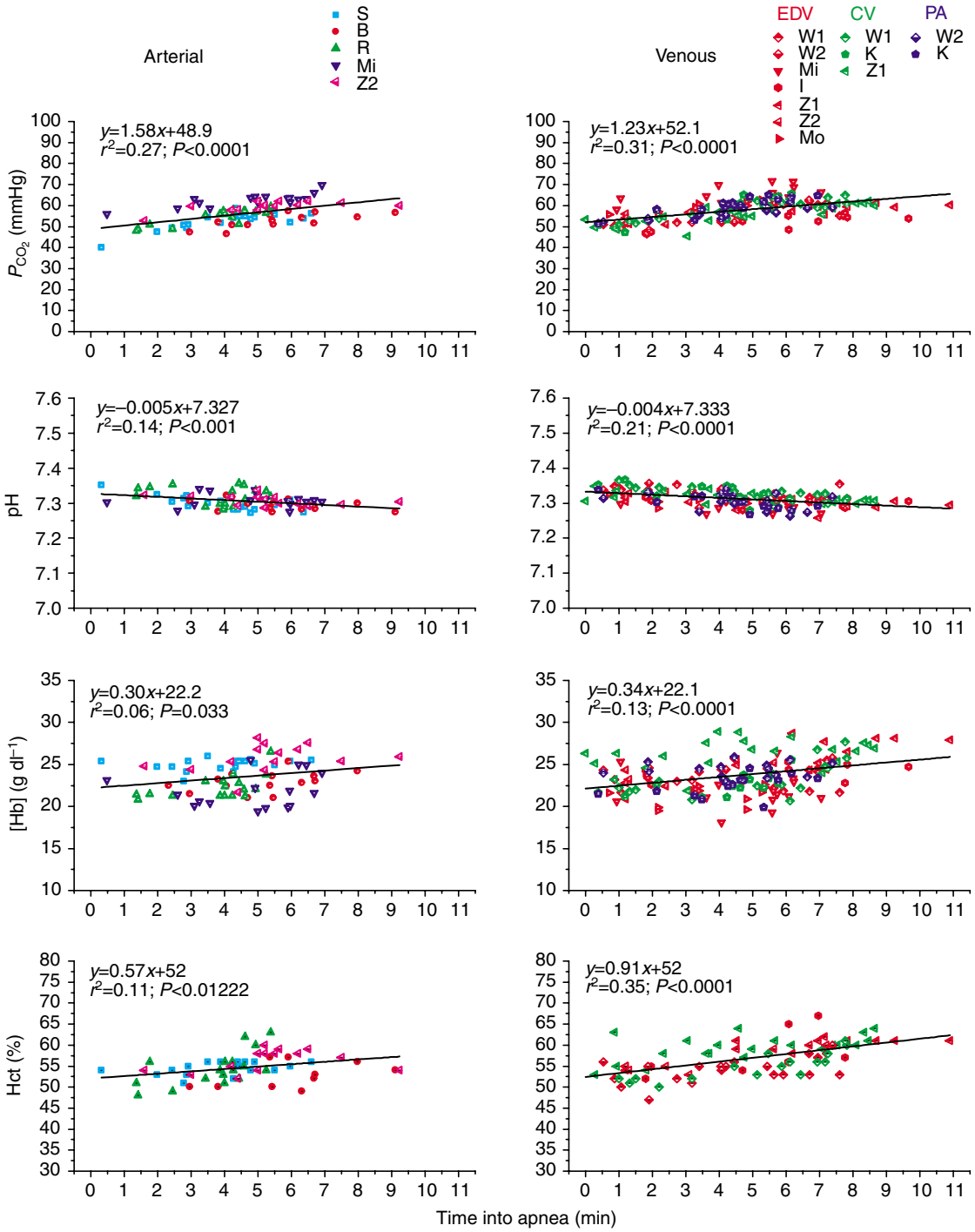

Fig. 4. Comparison of arterial and venous blood parameters (P_{CO_2}, pH, [Hb] and Hct) during apnea. See Table 2 for labels of individual seals.

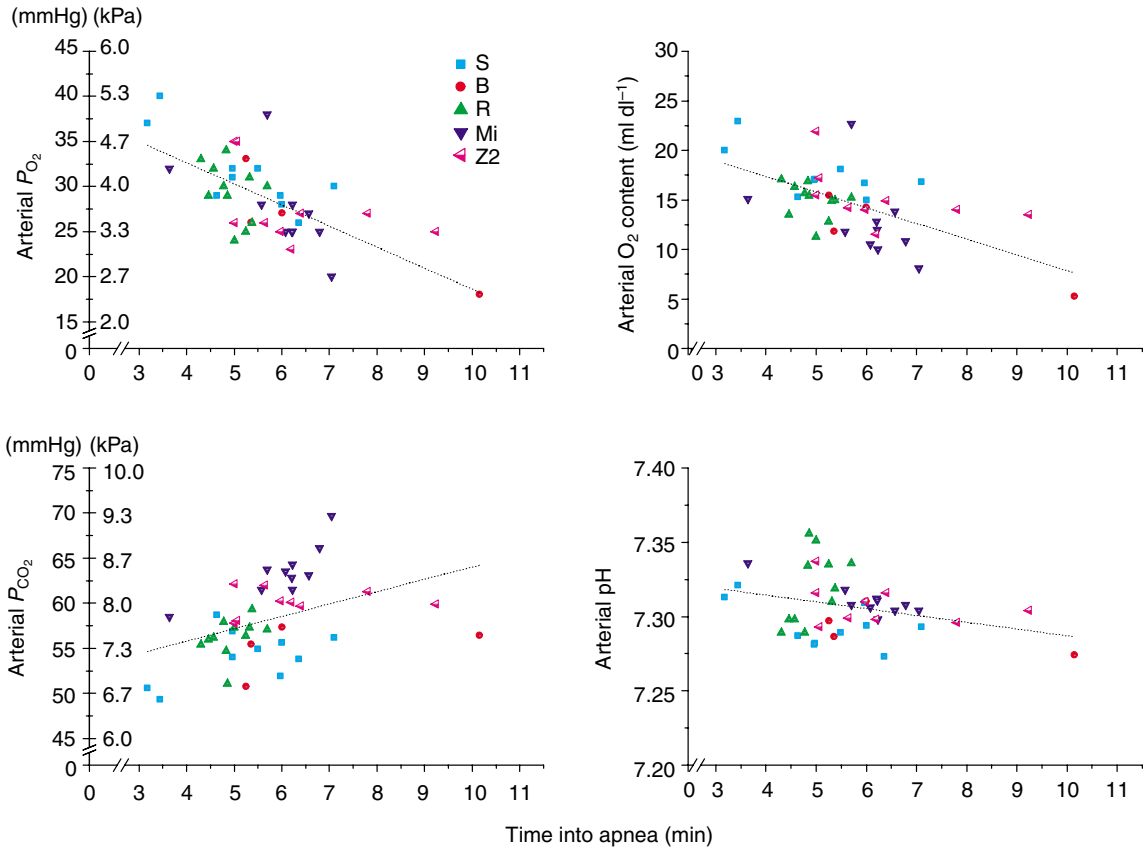

Fig. 5. Arterial end-of-apnea blood values as a function of apnea duration. Samples were drawn within the last minute of apnea. Dotted lines have been added as a visual aid only, to indicate trends, and do not imply any statistical significance. See Table 2 for labels of individual seals.

technical difficulties of successful blood sample collection in these studies, this approach was necessary.

Lastly, we concluded that it was appropriate to calculate O_2 contents from the [Hb] of a given sample and its percent saturation given by the i-STAT analyzer, based on the regression analysis of calculated *vs* empirical results from one experiment (y=1.003x+1.1, r^2=0.90, P<0.0001, N=24). The use of calculated values has been accepted in a previous study (Qvist et al., 1986) and was necessitated in the present study by the unavailability of the borrowed Tucker chamber and oxygen electrode in remaining experiments.

Eupnea

Due to the characteristic irregular, intermittent breathing pattern of phocid seals (Bartholomew, 1954; Blackwell and Le Boeuf, 1993; Harrison and Kooyman, 1968; Kenny, 1979), the eupneic blood values recorded from the elephant seals in this study were variable, as found in previous studies (Tables 3, 4). The data in Table 3 demonstrate that similar ranges and variation existed within individuals. In general, arterial P_{O_2} values were lower, and P_{CO_2} values higher, than those of terrestrial animals at rest (Taylor et al., 1987) and were within the same range as eupneic values of actively diving seals (Kooyman et al., 1980; Qvist et al., 1986). Differences in respiratory rates, tidal volumes, ventilation/perfusion matching in the lungs and splenic contraction may contribute to such variation.

The blood O_2 store

The magnitude of the available blood O_2 store is dependent on blood volume, Hb content and the pre-apneic Hb saturation. The circulating Hb content and the Hct used in the calculation of V_B from V_P are affected by the degree of splenic contraction (Ponganis et al., 1993; Qvist et al., 1986). In determining V_B from V_P, we used the highest Hct measured during the rest apneas of a given seal, an approach (Ponganis et al., 1993) that was confirmed with labeled red blood cell and plasma volume determinations in Weddell seals (Hurford et al., 1996). This technique may result in an underestimation of V_B and the blood O_2 store if splenic contraction is incomplete during rest apneas. However, Hcts used in the V_B calculation (Table 1) were >55% in five of seven seals and ⩾60% in four seals. Given the similarity between V_B results in this study and values in the literature as noted above (Simpson et al., 1970; Thorson and Le Boeuf, 1994), we believe this approach yields reasonable estimates of V_B in the seals.

Calculation of the available pre-apneic blood O_2 stores assumed blood volume fractions of 0.33 arterial and 0.67 venous and that all blood O_2 was available for consumption. The mean arterial and venous pre-apneic O_2 contents (y-intercepts in Fig. 3) were 27.2 and 26.0 ml O_2 dl^{-1}, respectively. With these assumptions and variables, the mean mass-specific blood O_2 store prior to rest-associated apnea would be 52 ml O_2 kg^{-1}. In contrast to this value for rest apnea, it is quite possible that the available blood O_2 store prior to a dive may be higher, since

Table 4. *Range of resting eupneic blood values from previous studies*

Sampling site	P_{O_2} (mmHg)	O_2 content (ml dl^{-1})	P_{CO_2} (mmHg)	[Hb] pH	(g dl^{-1})	*N*
Arterial						
Weddell[1]	60–77	37–58	7.28–7.32	7		
Weddell[2]	63–88	39–48	7.35–7.40	17.4±1.2	12	
Weddell[3]	78.1±12.9	18.8±1.42	42.5±1.63	7.37±0.010	15.1±1.10	19–22
Harbor[4]	60–88	42–55	7.32–7.38			
Northern elephant[5]	28–39					
Venous						
Harbor[4]	43–57	53–66	7.24–7.33			
Northern elephant[5]	28–39					

[1](Zapol et al., 1979). *N*=7 measurements from four seals; simulated dives.
[2](Kooyman et al., 1980). *N*=12 measurements from four seals; resting unrestrained on hut floor. (*N*=46 for Hb.)
[3](Qvist et al., 1986). Four seals; unrestrained, resting at surface.
[4](Kerem and Elsner, 1973). Restrained.
[5](Elsner et al., 1964). Four seals; forced dives.

ventilatory and heart rates are higher during surface intervals between dives than they are during rest apneas (Andrews et al., 2000; Andrews et al., 1997). These differences could result in higher P_{O_2} values, lower P_{CO_2} values, higher Hb saturation and greater O_2 contents for a given [Hb] in both the arterial and venous reservoirs prior to a dive. For example, in free-diving Weddell seals, the highest arterial P_{O_2}s recorded were always from the short rest period prior to a dive and were higher than mean resting values (Kooyman et al., 1980; Qvist et al., 1986). The highest pre-apneic arterial and venous O_2 contents in this study were 35.0 and 32.9 ml O_2 dl^{-1}, respectively. These were from blood samples that were 97% and 90% saturated. These levels, which are approaching the maximum oxygen contents possible for a [Hb] near 27 g dl^{-1}, would result in a pre-dive blood O_2 store of 66 ml O_2 kg^{-1}.

The initial arterial and venous O_2 contents used in the calculation of the pre-apneic blood O_2 store differed by only 1.2 ml O_2 dl^{-1}. Because of this small difference, the magnitude of the blood O_2 store is not affected by the degree of splenic contraction or relaxation at the start of an apnea. Even if 50% of the venous blood volume (66 ml blood kg^{-1}) were stored with an arterial O_2 content in the spleen, this would add less than 1 ml O_2 kg^{-1} to the calculated blood O_2 store in this study. Therefore, in this situation, the contraction state of the spleen has little effect on the total blood O_2 store.

Apnea

The apneic profiles of P_{O_2}, O_2 content, pH, P_{CO_2}, [Hb] and Hct (Figs 1–4) describe the general pattern of change in these parameters among several seals in relation to time into apnea. Although the rate of change in these parameters cannot be calculated for an individual seal or apnea, the data nevertheless provide several valuable observations.

First, arterial P_{O_2} values are indistinguishable from venous data after the first minute of apnea. In addition, initial venous O_2 content is nearly as high as the arterial value prior to the start of the breath-hold. These data confirm Scholander's prediction (Scholander, 1940) that the venous blood is highly saturated in seals, and reinforce the concept of a venous O_2 reservoir (Elsner, 1969; Elsner et al., 1964). It would appear that a small apneic lung volume (due to exhalation prior to the breath-hold) and probable lung atelectasis minimize any contribution of the lung to the total body O_2 store during these breath-holds.

Second, Elsner and colleagues' (Elsner, 1969; Elsner et al., 1964) observation that hepatic sinus O_2 contents were greater than aortic values during forced submersions is also supported by the general patterns of decline in P_{O_2} and O_2 content at the EDV, CV and PA sites (Fig. 2) and by the two paired analyses (HS *vs* PA; EDV *vs* PA) conducted in the current study. Although limited by sample size, these findings support the concept that depletion of lung O_2 and mixture of less oxygenated blood from the brain and heart with blood from the hepatic sinus will result in a lower O_2 content in the pulmonary artery and aorta during apnea.

Third, the relatively high initial Hb concentrations and O_2 contents during apneas in this study, and the small change in [Hb] throughout apneas, are similar to previous findings during sleep apnea (Castellini et al., 1986) and support the concept that short eupneic intervals do not provide sufficient time for the large spleen of a seal (Bryden and Lim, 1969; Ponganis et al., 1993; Qvist et al., 1986) to fully re-expand and significantly lower hematocrit and [Hb] between apneic periods. Castellini (Castellini, 1994) noted that Hct remained elevated during sequential apnea–eupnea cycles (as were characteristic of the seals in our study), and that it was necessary for the seal to be awake and breathing for several minutes after these cycles before Hct would decrease significantly. Two other studies have documented that splenic re-expansion times observed after dives (6–9 min half-times) (Hurford et al., 1996) and forced submersions (18–22 min for complete re-expansion) (Thornton et al., 2001) are long in comparison with eupneic intervals. The eupneic phases in our study generally lasted only a few minutes, with much of that time characterized by intermittent, slow breaths and usually with the seal still apparently asleep. The relatively high [Hb]s throughout apnea–eupnea cycles during both diving activity (Castellini et al., 1988) and sleep apnea suggest that the spleen is predominantly contracted during these activities, with only minor variation in the degree of expansion

or contraction during eupnea and apnea, respectively. In addition, as already discussed, the magnitude of the blood O_2 store in these seals is not significantly affected by the degree of splenic contraction or relaxation. However, it should be noted that any further contraction of an incompletely contracted spleen during apnea would presumably contribute to the maintenance of hepatic sinus O_2 content during apnea.

Lastly, the minimal change in pH throughout these apneas is presumably secondary to the modest increase in P_{CO_2} (Fig. 4) and is consistent with the lack of lactate accumulation previously documented during sleep apnea of seals (Castellini, 1994; Castellini et al., 1986). Given the low range of arterial P_{O_2} during these apneas, the lack of evidence for significant glycolysis again emphasizes the ability of these animals to continue to function in what would be considered extreme hypoxemia in other mammals.

Depletion of blood O_2 stores

During apnea, O_2 contents declined linearly in both the arterial and venous compartments, at rates of 2.3 and 2.0 ml O_2 dl^{-1} min^{-1}, respectively (Figs 1, 2). These values represent overall depletion rates, not instantaneous blood O_2 consumption rates throughout the apnea. The depletion rates found during rest apnea in this study are greater than those found during forced submersions of similarly sized seals, or during free dives of larger seals. For example, Scholander found that during forced submersions of grey seals (*Halichoerus grypus*) and hooded seals (*Cystophora cristata*), arterial oxygen was depleted at a constant rate of 1.3 and 1.6 ml O_2 dl^{-1} min^{-1}, respectively (Scholander, 1940). Analyses of forced submersion data revealed arterial O_2 depletion rates of 1–2 ml O_2 dl^{-1} min^{-1} in harbor seals (Kerem and Elsner, 1973), and venous and arterial O_2 depletion rates of 1.0 and 1.3 ml O_2 dl^{-1} min^{-1} in elephant seals (Elsner et al., 1964; Elsner, 1969). In the only study that measured O_2 content in free-diving seals (Weddell seals), Qvist et al. found that arterial O_2 content decreased by 0.8 ml O_2 dl^{-1} min^{-1} (Qvist et al., 1986). In comparison with the data from forced submersions, the rapid rates of blood O_2 depletion observed in the current study are consistent with higher cardiac outputs during rest apneas (Ponganis et al., 2006) than during forced submersions (Blix et al., 1983). This is consistent with the classic concept that heart rate and organ perfusion control the rate of blood O_2 depletion during apnea.

With a mean pre-apneic blood O_2 store of 52 ml O_2 kg^{-1} (calculated using the intercepts of arterial and venous O_2 content regressions in Fig. 3B), the mean blood oxygen depletion rates in Fig. 3B would result in consumption of 56% of the initial blood O_2 store by the end of a typical 7-min apnea. This depletion of blood O_2 would contribute 4.2 ml O_2 kg^{-1} min^{-1} to total body metabolic rate during apnea. This estimated blood O_2 contribution to apneic metabolic rate appears reasonable in that oxygen consumption measured in 10–22-month-old juvenile northern elephant seals resting at the surface in a laboratory tank was 4.46±0.3 ml O_2 kg^{-1} min^{-1} (Webb et al., 1998). In addition, Thorson and Le Boeuf found that oxygen consumption, averaged over both the apneic and eupneic intervals in young elephant seals, ranged from 2 to 6 ml O_2 kg^{-1} min^{-1}, with a value near 3 ml O_2 kg^{-1} min^{-1} for a 7 min apnea (Thorson and Le Boeuf, 1994). This large blood O_2 contribution to apneic metabolic rate is again consistent with the maintenance of cardiac output during rest-associated apneas (Ponganis et al., 2006) and reinforces the hypothesis that most metabolic processes continue aerobically during these breath-holds.

End of apnea and hypoxemic tolerance

Table 5 compares the results from our study with previously published data from rest apneas, forced submersions, and free-diving in different seal species. The lowest P_{O_2} values

Table 5. *Comparison of end-of-apnea data in the literature*

		Present study[1]	Kooyman et al., 1980[2]	Elsner, 1964[3]	Kerem and Elsner, 1973[4]	Zapol et al., 1979[5]	Qvist et al., 1986[6]
		Elephant seal (juvenile); rest-associated apnea	Weddell seal; (adult); rest-associated apnea	Elephant seal forced submersion	Harbor seal; forced submersion	Weddell seal; forced submersion	Weddell seal; free-diving
P_{O_2}	Arterial	18–26	25	–	10±0.85	31.5±5	19.9±2.05
	Venous	15–31	–	–	2.6±1.06	–	–
P_{CO_2}	Arterial	57–70	55	–	103	59±6.5	50.5±1.17
	Venous	55–72	–	–	111	–	–
pH	Arterial	7.27–7.30	7.32	–	7.1	7.25±0.03	7.34±0.025
	Venous	7.26–7.31	–	–	7.0	–	–
O_2 content	Arterial	5.2–15.0	–	4.6	2.6	–	11.7±2.32
	Venous	4.80–16.7	–	6.6	1	–	–

[1]Range includes the lowest value (or highest, for P_{CO_2}) for each seal. *N*=5 seals for arterial values; 8 seals for venous.
[2]Values are endpoints from rest-apneas on the hut floor or in the water (values were the same). *N*=2 seals.
[3]*N*=4 seals.
[4]P_{O_2} values are means ± s.d. recorded at EEG hypoxia endpoint (*N*=17 trials). Remaining values are from single experiments.
[5]Means ± s.d. from 6 trials, 4 seals.
[6] Means ± s.d. from long dives (>17 min). *N*=4 samples. Note that this is the only study that incorporated exercise.

(18 mmHg arterial; 15 mmHg venous) during rest apneas of these elephant seals are greater than those (10 mmHg arterial and 2.5 mmHg venous) in extreme forced submersions of harbor seals (Kerem and Elsner, 1973), but are similar to arterial and end tidal values during rest apneas and free dives of Weddell seals (Kooyman et al., 1980; Ponganis et al., 1993; Qvist et al., 1986). A similar pattern holds for O_2 content. The lowest arterial P_{O_2} values during rest-associated apneas of elephant seals are also lower than the end-tidal P_{O_2} of climbers on Mt. Everest without supplemental oxygen (35 mmHg) (West et al., 1983), and lower than the 25 mmHg P_{O_2} threshold typically associated with loss of consciousness in humans (Ferretti et al., 1991; Ferrigno and Lundgren, 2003). Several mechanisms may contribute to such hypoxemic tolerance. In addition to potential effects of hypercarbia on Hb-O_2 binding and the magnitude of cerebral blood flow, one possible explanation is the higher brain capillary density and shorter diffusion distances found in elephant seals vs. terrestrial animals (Kerem and Elsner, 1973). Another possibility is that the brains of seals may contain high concentrations of the recently discovered oxygen-binding protein neuroglobin (Burmester et al., 2000), although this has yet to be studied.

Given that juvenile elephant seals spend up to 70% of their sleep in apnea (Blackwell and Le Boeuf, 1993), it appears that these animals spend a significant portion of their time in what would be considered a hypoxemic state in other mammals. For example, tolerance of these low oxygen tensions would make it possible for up to 88% of the initial blood O_2 store to be extracted during an 11-min apnea (as exhibited by one of our seals). Yet, despite such low levels of oxygen tension, which are necessary to allow dissociation of O_2 from Hb for utilization of the blood O_2 store, there is no evidence of significant glycolysis or lactate accumulation (Castellini et al., 1986; Castellini, 1994). The minimal pH changes observed in our study, which were attributable to CO_2 accumulation during apnea, support those previous findings and emphasize the ability of these animals to maintain aerobic metabolic function, even at low P_{O_2} values.

Data from arterial blood samples drawn within the last minute of apnea were plotted against apnea duration (Fig. 5) to discern if there were any critical parameters that would trigger the end of apnea. The endpoint values were variable, both within and among individuals, indicating that there were no definable thresholds associated with the resumption of breathing. Qualitatively, end-of-apnea P_{O_2} and O_2 contents were lower with longer apneas, varying by as much as 22 mmHg or 18 ml dl^{-1}, respectively. End-of-apnea P_{CO_2} showed an increasing trend as apnea duration increased, varying between 49 and 70 mmHg, while pH fluctuated by less than 0.1 pH unit. In previous studies (Milsom et al., 1996; Stephenson, 2005), it has been suggested that CO_2 may play a primary role in the drive to breathe, but such investigations of respiratory control were beyond the scope of the present project. Our results serve only to demonstrate that end-of-apnea levels are variable in the blood and that there is no apparent threshold associated with the end of apnea.

Conclusions

The rest-associated apneas of the juvenile elephant seals in this study lasted between 3.1 and 10.9 min. The mean pre-apneic venous O_2 content of 26.0 ml O_2 dl^{-1} was almost equivalent to the mean arterial value of 27.2 ml O_2 dl^{-1}. During apnea, blood oxygen was depleted at an average rate of 2.3 ml O_2 dl^{-1} min^{-1} in the arterial system and 2.0 ml O_2 dl^{-1} min^{-1} in the venous system. During a typical 7-min breath-hold, blood O_2 depletion was calculated to contribute 4.2 ml O_2 kg^{-1} min^{-1} to total body metabolic rate during apnea and to consume 56% of the initial blood O_2 store (52 ml O_2 kg^{-1}). These O_2 depletion rates are approximately twice those observed during forced submersion and are consistent with maintenance of cardiac output during rest-associated apneas. Blood P_{O_2} declined exponentially during apnea, with arterial and venous values becoming similar after the first minute of the breath-hold. The lowest P_{O_2} values recorded were 18 mmHg (arterial) and 15 mmHg (venous), demonstrating remarkable hypoxemic tolerance in these seals even during rest-associated apneas. Despite such low O_2 tensions, changes in pH were consistent with increased P_{CO_2} but showed no evidence of a metabolic acidosis and lactate accumulation. All these findings are consistent with the maintenance of aerobic metabolism despite low P_{O_2} during these apneas. Such hypoxemic tolerance is necessary in order to allow O_2 dissociation from Hb and utilization of the blood O_2 store during apnea.

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