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O_2 store management in diving emperor penguins

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SUMMARY

In order to further define O_2 store utilization during dives and understand the physiological basis of the aerobic dive limit (ADL, dive duration associated with the onset of post-dive blood lactate accumulation), emperor penguins (*Aptenodytes forsteri*) were equipped with either a blood partial pressure of oxygen (P_{O_2}) recorder or a blood sampler while they were diving at an isolated dive hole in the sea ice of McMurdo Sound, Antarctica. Arterial P_{O_2} profiles (57 dives) revealed that (a) pre-dive P_{O_2} was greater than that at rest, (b) P_{O_2} transiently increased during descent and (c) post-dive P_{O_2} reached that at rest in 1.92±1.89 min (N=53). Venous P_{O_2} profiles (130 dives) revealed that (a) pre-dive venous P_{O_2} was greater than that at rest prior to 61% of dives, (b) in 90% of dives venous P_{O_2} transiently increased with a mean maximum P_{O_2} of 53±18 mmHg and a mean increase in P_{O_2} of 11±12 mmHg, (c) in 78% of dives, this peak venous P_{O_2} occurred within the first 3 min, and (d) post-dive venous P_{O_2} reached that at rest within 2.23±2.64 min (N=84). Arterial and venous P_{O_2} values in blood samples collected 1–3 min into dives were greater than or near to the respective values at rest. Blood lactate concentration was less than 2 mmol l^{-1} as far as 10.5 min into dives, well beyond the known ADL of 5.6 min. Mean arterial and venous P_{N_2} of samples collected at 20–37 m depth were 2.5 times those at the surface, both being 2.1±0.7 atmospheres absolute (ATA; N=3 each), and were not significantly different. These findings are consistent with the maintenance of gas exchange during dives (elevated arterial and venous P_{O_2} and P_{N_2} during dives), muscle ischemia during dives (elevated venous P_{O_2}, lack of lactate washout into blood during dives), and arterio-venous shunting of blood both during the surface period (venous P_{O_2} greater than that at rest) and during dives (arterialized venous P_{O_2} values during descent, equivalent arterial and venous P_{N_2} values during dives). These three physiological processes contribute to the transfer of the large respiratory O_2 store to the blood during the dive, isolation of muscle metabolism from the circulation during the dive, a decreased rate of blood O_2 depletion during dives, and optimized loading of O_2 stores both before and after dives. The lack of blood O_2 depletion and blood lactate elevation during dives beyond the ADL suggests that active locomotory muscle is the site of tissue lactate accumulation that results in post-dive blood lactate elevation in dives beyond the ADL.

Key words: aerobic dive limit, blood sampler, emperor penguin, hemoglobin, lactate, nitrogen, oxygen electrode, oxygen store, shunt.

INTRODUCTION

The concept of an aerobic dive limit (ADL) is central to most eco-physiological models of foraging behavior in marine mammals and diving birds. Although routinely calculated (but rarely measured), the ADL has remained a physiological black box. Conceptually, it should be dependent on the rate, pattern and magnitude of O_2 store depletion during dives. However, the management and utilization of O_2 stores during diving may vary in different species or situations due to (a) differences in the magnitude and distribution of O_2 stores and (b) differences in the intensity of physiological responses underlying the rate of O_2 store depletion during a dive. The ADL has been defined as the dive duration associated with the onset of post-dive blood lactate accumulation, and it has been proposed that the lactate accumulation at the ADL is secondary to localized tissue O_2 depletion and increased glycolysis, most probably in active but ischemic locomotory muscle (Kooyman and Ponganis, 1998). On the other hand, less-than-expected decreases in muscle O_2 saturation during dives of Weddell seals (*Leptonychotes weddellii*) have led to the suggestion that muscle blood flow persists during dives (Guyton et al., 1995), and to the hypothesis that maintenance of muscle blood flow during dives allows for the matched parallel depletion of blood and muscle O_2 stores (Davis and Kanatous, 1999). The primary difference between these two different ADL scenarios is dependent on the isolation of working muscle from the blood O_2 store during a dive.

Therefore, in order to increase our understanding of O_2 store management and the physiological basis of the ADL, we reviewed blood oxygen partial pressure (P_{O_2}) profiles and analyses of blood samples from diving emperor penguins (*Aptenodytes forsteri*) for findings indicative of blood flow patterns and the utilization of O_2 stores during dives. Emperor penguins diving at an isolated dive hole are particularly appropriate for such investigations because the ADL has been determined by post-dive blood lactate measurements to be 5.6 min, and neither the respiratory nor the blood O_2 stores are depleted at the ADL (Ponganis et al., 1997; Ponganis et al., 2007; Stockard et al., 2005). For dive durations beyond the 5.6 min ADL, post-dive blood lactate concentrations are elevated.

We had two primary hypotheses. First, we hypothesized that the maintenance of gas exchange with the large respiratory O_2 store in emperor penguins contributes to significant O_2 transfer from the respiratory system to the blood during dives. Second, we hypothesized that muscle is isolated from the blood O_2 store during dives, i.e. muscle blood flow and blood-to-muscle O_2 transfer stop during dives. In particular, we reasoned that blood P_{O_2} profiles during dives should be a reflection of (a) pulmonary gas exchange, (b) a reduction in cardiac output secondary to the bradycardia of diving, and (c) changes in blood O_2 extraction due to the reduction/redistribution of organ blood flow secondary to both the fall in cardiac output and peripheral vasoconstriction during dives.

MATERIALS AND METHODS

General approach, catheterizations and recorders

In the austral springs of 2001, 2003–2005 and 2007, non-breeding emperor penguins (*Aptenodytes forsteri* Gray, 20–30 kg in body mass) were captured on the sea ice of McMurdo Sound or at Terra Nova Bay near Cape Washington (74°36′, 165°24′). They were maintained at the corralled isolated dive hole of the Penguin Ranch in McMurdo Sound (77°41′, 165°59′) for 6 weeks as in past studies (Kooyman et al., 1992; Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003), and then released at the McMurdo Sound ice edge. Blood-sampling catheters or P_{O_2} electrodes/thermistors (Licox C1.1 Revoxode; Integra LifeSciences, Plainsboro, NJ, USA; model 554, Yellow Springs Instruments, Yellow Springs, OH, USA) were inserted percutaneously into the aorta or vena cava of emperor penguins under general isoflurane anesthesia as described previously (Ponganis et al., 2007; Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003). In addition, in two birds in 2007, successful femoral artery catheterization was achieved with a 4.5 in, 20 g catheter (Arrow International, Reading, PA, USA); only a P_{O_2} electrode was inserted through this small catheter. The birds were also equipped with a custom-made P_{O_2}/temperature recorder (UFI, Morro Bay, CA, USA) and an Mk9 time depth recorder (TDR, Wildlife Computers, Redmond, WA, USA) as previously described (Stockard et al., 2005). After overnight recovery from anesthesia, birds were allowed to dive at the isolated dive hole. After 1–2 days of diving and data collection, catheters, probes and recorders were removed under general anesthesia. Data from the recorders were downloaded to a personal computer and analyzed with Excel (Microsoft, Redmond, WA, USA), Origin (Origin Lab., Northampton, MA, USA) and SPSS (SPSS Inc., Chicago, IL, USA) software. As previously reviewed (Ponganis et al., 2007), all P_{O_2} values were corrected to 38°C for construction of the P_{O_2} profiles. In the two birds without thermistors in 2007, blood temperature was assumed to be 38°C.

All partial pressures are expressed, as measured, in mmHg (7.5 mmHg=1 kPa). Means are expressed ±s.d. Significance was assumed at $P<0.05$. All procedures were approved under a UCSD Animal Subjects Committee protocol and US Antarctic Treaty Permit.

Blood sampler

The custom-designed blood sampler (1.25 kg, 24 cm×8.5 cm diameter) collected one sample per dive and consisted of an underwater housing, a programmable, microprocessor-based control board (UFI), a pressure transducer (Model 96, IC Sensors, Milpitas, CA, USA), a blood access port, and two solenoid valves (2W13W-1NR-A1C4, Snap Tite, Erie, PA, USA) which each controlled access to a syringe, one for waste (>3 times dead space volume) and one for the blood sample (Fig. 1). Collection of blood relied upon the pressure difference between the ambient pressure at depth and that inside the housing. The catheter port inside the housing was connected to the solenoid valves *via* high pressure Teflon tubing and swedgelock fittings. The sampler was programmed to obtain the sample after detection of a specified depth, or to obtain the sample when a specified time interval had elapsed while the bird was beneath a specified 'start-of-dive' depth threshold. The sampler was also programmed to delay activation for a minimum of 1 h after the sampler was mounted on a bird in order to avoid any effects secondary to restraint. The unit was retrieved by recapture/restraint of the bird when blood was visible in the external transparent stopcock connection between the catheter and blood sampler port (no. 91041 stopcock, Mallinckrodt, Glen Falls, NY, USA; 3.5 in extension tubing no. 53035, Medex, Dublin, OH, USA). Catheters for blood sampling were in the femoral artery, femoral vein or axillary vein. Lyophilized heparin (210-6, Sigma Aldrich, St Louis, MO, USA) was placed in the sample syringe (Becton Dickinson, Franklin Lakes, NJ, USA) to prevent clotting. Because the volume of the blood sample syringe was diluted by the dead space flush volume of its solenoid valve, analyses for blood gases, O_2 content and lactate concentration were performed on samples collected anaerobically from the undiluted blood in the Teflon tubing in the sampler. As in past blood sampler studies (Falke et al., 1985), nitrogen content and, in some cases, lactate concentration determined from the sample syringe were corrected for dilution by comparison of hematocrit (determined by microcentrifugation) or hemoglobin (Hb) concentration in the sample syringe *versus* that in the tubing. Blood samples were analyzed within 120 min after collection of the sample due to continued diving activity and the time required to recapture the bird and dismantle the blood sampler.

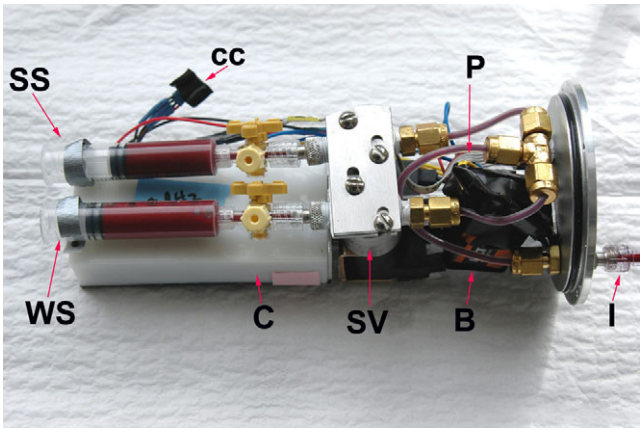

Fig. 1. Overhead view of the blood sampler after removal from underwater housing. B, 9 V battery; C, circuit board housing; cc, computer communication port; I, inlet; P, cable from bulkhead-mounted pressure transducer; SS, sample syringe; SV, solenoid valve; WS, waste syringe.

Blood sample analyses

In addition to collection of blood samples from birds at rest (Ponganis et al., 2007) and from diving birds, occasional blood samples were also manually collected from birds under anesthesia and from restrained birds during surface intervals. Blood gas (P_{O_2}, P_{CO_2} and pH) and lactate concentration analyses on these samples were conducted with a Series 200 i-STAT Portable Clinical Analyzer (CG4+ cartridge, Abbott Point of Care Inc., East Windsor, NJ, USA) at 37°C (Stockard et al., 2007). O_2 content was determined with a Tucker chamber technique (Models SI 782 O_2 meter and 1302 O_2 electrode, Strathkelvin, Motherwell, UK) (Tucker, 1967). Blood N_2 content was determined with the Van Slyke technique (Ponganis et al., 1999). The N_2 solubility coefficient (1.44 ml N_2 per 100 ml blood per atmosphere N_2) was determined in blood tonometered with ambient air or 100% N_2 at 38°C. Hb concentration was determined with a cyanmethemoglobin spectrophotometric technique (Stockard et al., 2007). Blood samples were analyzed within 10 min of collection. Blood gas, O_2 content, lactate concentration and P_{N_2} were stable for as long as 4 h at room temperature in the blood gas syringes (Model 4041, Sims Portex, Keene, NH, USA).

RESULTS

Behavior

For birds equipped with the P_{O_2} recorder, most dive durations and maximum depths have previously been described, and were similar

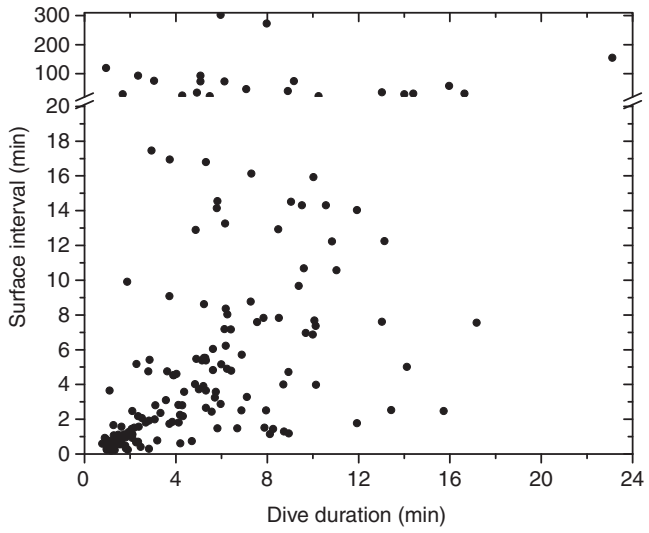

Fig. 2. The relationship of surface interval to dive duration of emperor penguins diving at the isolated dive hole.

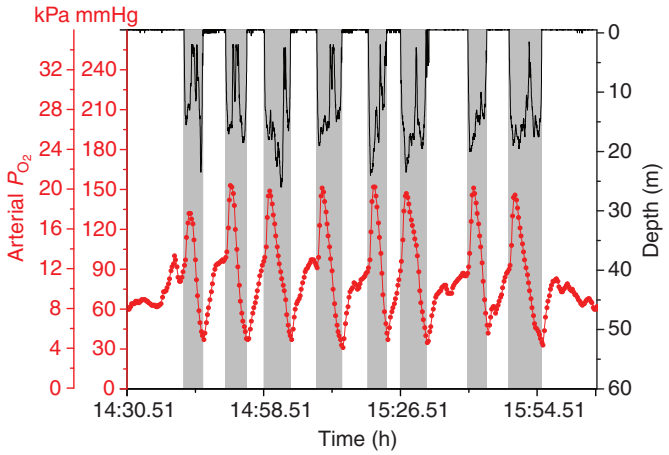

Fig. 3. Arterial P_{O_2} (red) and depth (black) profiles during dives of emperor penguin 10. Shaded area indicates diving.

to those in past studies at the isolated dive hole (Ponganis et al., 2007; Stockard et al., 2005). Additional arterial P_{O_2} profiles obtained from two birds in 2007 increased the number to 57 dives in five birds; venous profiles were from 130 dives in nine birds. Mean dive duration of all 187 dives with P_{O_2} recorders was 5.68±3.99 min; mean dive depth was 34±22 m. The relationship of surface interval to dive duration (Fig. 2) was similar to that previously reported for emperor penguins at sea (Kooyman and Kooyman, 1995; Wienecke et al., 2007).

Dive durations and maximum depths of penguins equipped with the blood sampler ranged from 3 to 12.8 min and 28 to 55 m, respectively. These depths and durations were within the range of dive durations exhibited by birds without such a large recorder. However, as previously observed in emperor penguins equipped with and without a Crittercam camera (Ponganis et al., 2000), the increased drag of the larger unit probably resulted in dives of shorter duration. Nonetheless, dive durations as long as 12.8 min did occur in birds equipped with the sampler. Arterial blood samples were obtained 2.1, 2.8 and 5.2 min into dives of 4.4, 10.1 and 10.5 min duration in one bird. Venous samples were obtained 1.1, 1.7, 2.2, 2.3, 3.2 and 10.5 min into dives of 7.3, 7.8, 4.1, 3.0, 6.6 and 12.8 min duration in four birds. In general, the number of samples obtained was limited by (1) the design of the sampler (one sample per dive and size of the unit), (2) the time required for filling the syringes at these shallow depths (1 min per syringe), (3) our inability to predict the duration of a dive when trying to obtain samples late in a dive, and (4) a frequent lack of interest in diving that occurred after a bird was restrained and equipped with the sampler but which resolved itself after removal of the sampler.

P_{O_2} profiles

Arterial P_{O_2} profiles during dives (see Fig. 3) revealed that (1) pre-dive P_{O_2} was usually elevated above the previously reported level (68 mmHg) for emperor penguins at rest (Ponganis et al., 2007), (2) P_{O_2} consistently increased during the early part of the dive, usually peaking at the end of the initial descent of the dive, (3) at similar depths (Fig. 4) these peak arterial P_{O_2} values were distinctly less than the previously measured peak air-sac P_{O_2} values (Stockard et al., 2005), (4) arterial P_{O_2} declined as the dive progressed, resulting in final P_{O_2} values similar to those previously reported for air-sac and venous P_{O_2} (Fig. 5), and (6) prior to the next dive, post-dive arterial P_{O_2} reached 68 mmHg within 1.92±1.89 min (N=53).

Venous P_{O_2} profiles during dives (Figs 6 and 7) were remarkable for their variable patterns. During the pre-dive period, venous P_{O_2} often fluctuated; it decreased prior to the start of the dive in 51% of dives (see Fig. 6 for an example). Pre-dive venous P_{O_2} ranged from 21 to 82 mmHg (mean 44±12 mmHg), and initial dive P_{O_2} ranged from 24 to 79 mmHg (mean 45±12 mmHg). In 61% of dives, pre-dive venous P_{O_2} was greater than that (41 mmHg) of birds at rest (Ponganis et al., 2007). Dive duration was very weakly, but significantly, related to each of these variables (Fig. 8; pre-dive P_{O_2}: y=0.113x+1.18, R^2=0.08, P<0.05; initial P_{O_2}: y=0.099x+1.18, R^2=0.06, P<0.05). In 90% of dives, the venous P_{O_2} increased transiently (within the first 3 min in 78% of dives) with a maximum

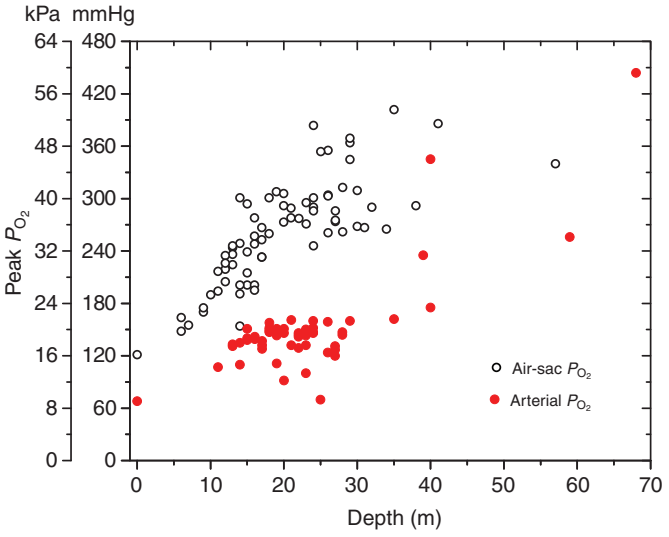

Fig. 4. The relationship of peak air-sac (Stockard et al., 2005) and arterial P_{O_2} to the depths at which they occurred in emperor penguins diving at the isolated dive hole.

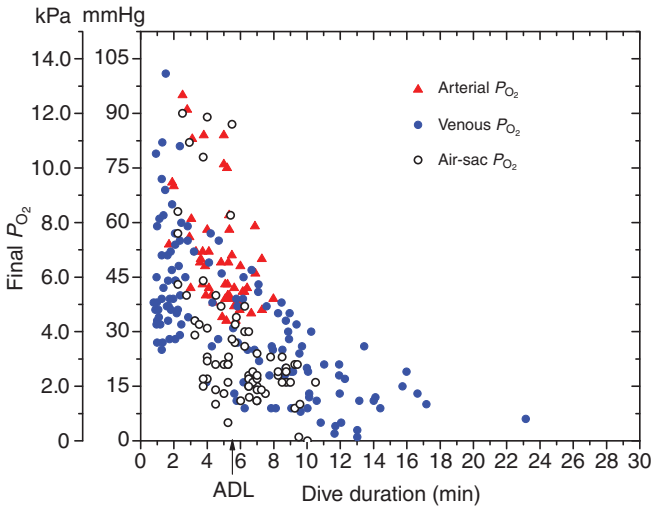

Fig. 5. The relationship of the final P_{O_2} of a dive to dive duration in the air sac (Stockard et al., 2005), aorta [this study and Ponganis et al. (Ponganis et al., 2007)] and vena cava (Ponganis et al., 2007). ADL, aerobic dive limit.

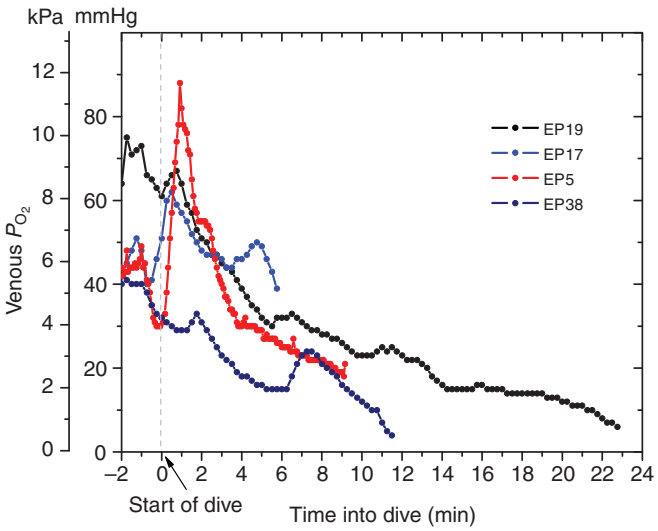

Fig. 7. Comparison of venous P_{O_2} in four emperor penguins (EP5, 17, 19, 38) with dives of different durations. Decreases in venous P_{O_2} occurred prior to diving. Transient elevations in venous P_{O_2} are evident at various points in each dive; the initial elevations occurred within the first 2–3 min of these dives.

P_{O_2} of 53±18 mmHg (range 28–129 mmHg) and a mean increase in P_{O_2} of 11±12 mmHg (range 2–76 mmHg). Small transient increases could occur later and more than once during a dive (see Fig. 7). Maximum venous P_{O_2} during a dive was >68 mmHg in 15% of dives, and >41 mmHg in 71% of dives; again, these levels correspond to the arterial and venous P_{O_2} values of emperor penguins at rest (Ponganis et al., 2007). Regression analysis revealed that dive duration was not significantly related to the maximum venous P_{O_2} during a dive. During short dives, the increase in venous P_{O_2} could result in final venous P_{O_2} values that were greater than the start-of-dive venous P_{O_2} [see figure 2 in Ponganis et al. (Ponganis et al., 2007)].

After 84 dives, post-dive venous P_{O_2} reached 41 mmHg prior to the next dive; this required 2.23±2.64 min. After a 23.1 min dive, venous P_{O_2} reached this value in less than 2 min. Regression analysis revealed that the relationship of prior dive duration to the time for venous P_{O_2} to recover to 41 mmHg was not significant. The relationship of surface interval to the time to reach a venous P_{O_2} of 41 mmHg was also not significant.

In the post-dive period, venous P_{O_2} initially continued to decline below the final dive value after 48% of dives. The time for post-dive venous P_{O_2} to return to 41 mmHg was not significantly longer than that required for arterial P_{O_2} to return to 68 mmHg, but both intravascular return times were significantly longer than the 0.92±0.44 min [N=73, data from Stockard et al. (Stockard et al., 2005)] required for air-sac P_{O_2} to return to the level of birds at rest (ANOVA, F=9.308, P<0.05; Tukey HSD *post-hoc* analysis, P<0.05).

Fig. 9 represents a comparison of typical air-sac, arterial and venous P_{O_2} profiles and a heart rate profile of different birds for shallow (<50 m) dives of approximately 8 min duration. The data are from this and prior studies (Meir et al., 2008; Stockard et al., 2005).

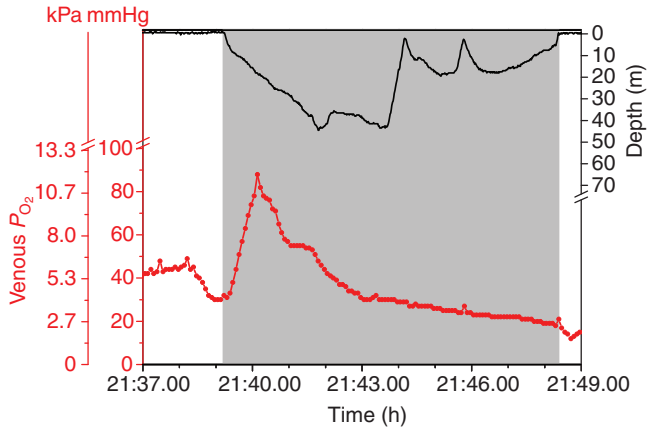

Fig. 6. Venous P_{O_2} and depth profiles during a 9.2 min dive of emperor penguin 5. Shaded area indicates dive. Venous P_{O_2} declined both before and after this dive. The peak P_{O_2} during the dive was 90 mmHg; it occurred during descent at less than 2 min into the dive.

Blood analyses in penguins at rest and during non-diving periods

Arterial and venous N_2 partial pressures (P_{N_2}) in emperor penguins at rest were 0.86±0.04 atmospheres absolute (ATA; N=3) and 0.85±0.06 ATA (N=9), respectively. Blood gas (P_{O_2}, P_{CO_2}, pH), O_2 content and lactate concentrations of these birds at rest have previously been reported (Ponganis et al., 2007).

Venous P_{O_2} and P_{CO_2} from the brachial vein (wing) of a restrained penguin during a post-dive surface interval were 81 and 38 mmHg, respectively. During anesthesia of one bird on 100% O_2, near-simultaneous sampling of the brachial artery and brachial vein yielded P_{O_2} values of 247 and 260 mmHg, respectively. Hemoglobin concentrations of three penguins during anesthesia and at rest were 16.5±0.6 and 16.4±0.8 g dl^{-1}, respectively.

Blood analyses in diving penguins

Blood gas (P_{O_2}, P_{CO_2}, pH) and O_2 content were measured in two arterial and three venous samples obtained during dives (Table 1). It is notable that arterial and venous P_{O_2} values at between 1 and

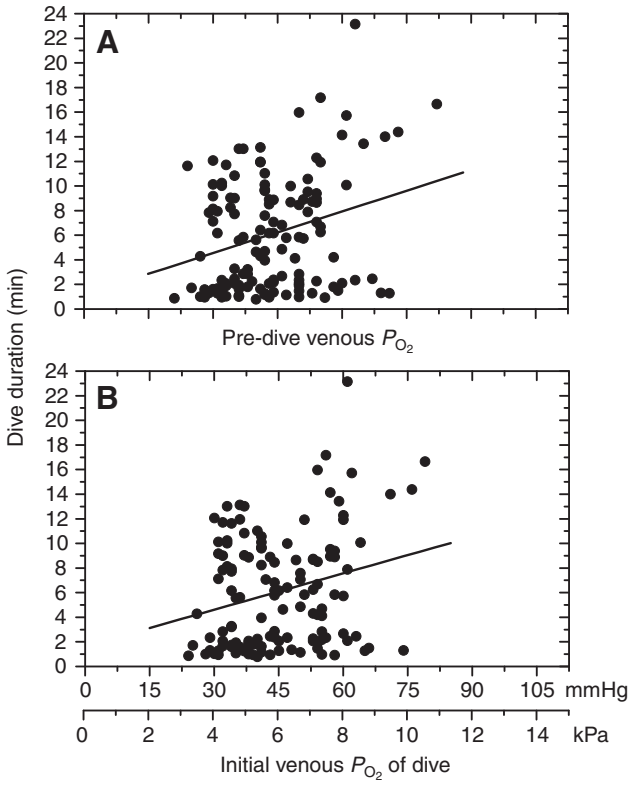

Fig. 8. The relationship of dive duration to pre-dive venous P_{O_2} and initial venous P_{O_2}. (A) Pre-dive P_{O_2}: y=0.113x+1.18, R^2=0.08, P<0.05; (B) initial P_{O_2}: y=0.099x+1.18, R^2=0.06, P<0.05.

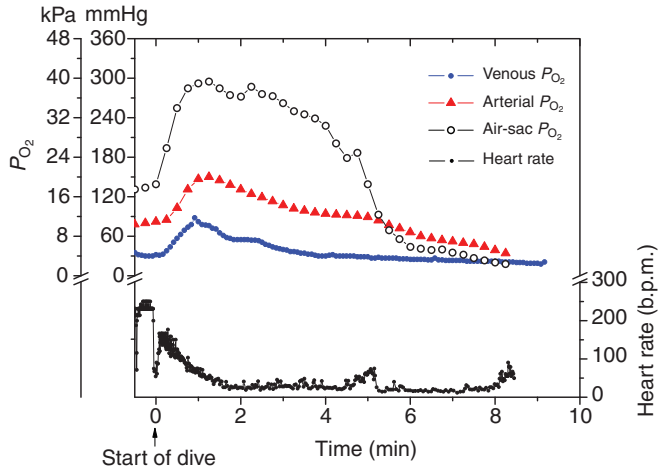

Fig. 9. Air-sac P_{O_2}, arterial P_{O_2}, venous P_{O_2} and heart rate profiles during shallow (<50 m), approximately 8 min dives of four emperor penguins. Elevations in air-sac, arterial and venous P_{O_2} values and in heart rate all occur within the first 2 min of the dives. Air sac, bird 7 (Stockard et al., 2005); arterial, bird 10; venous, bird 5; heart rate, bird 37 (Meir et al., 2008). b.p.m., beats per min.

3 min into the dives were above or near the respective values of penguins at rest. Blood lactate concentration was less than 2.0 mmol l^{-1} as far as 10.5 min into a dive (Fig. 10). Mean arterial P_{N_2} at depths of 25–32 m at 2.1–5.2 min into a dive was elevated at 2.1±0.7 ATA (N=3). Venous P_{N_2} at 20–37 m depth at 1.7–3.2 min into a dive was 2.1±0.7 ATA (N=4). These arterial and venous P_{N_2} values were not significantly different (Student's two sample t-test).

Hb concentration during dives of the same three penguins sampled for Hb under anesthesia and at rest was 16.3±0.7 g dl^{-1}. The Hb concentrations during anesthesia, at rest and during dives were not significantly different (ANOVA, F=5.14, P=0.94).

DISCUSSION

Maintenance of gas exchange during dives

The initial transient increases in arterial P_{O_2} profiles during dives reflected the compression hyperoxia previously observed in the air sacs of diving emperor penguins and confirmed the maintenance of pulmonary gas exchange and transfer of O_2 from the respiratory store into the blood O_2 store during the dive. As a result, during some dives, final arterial P_{O_2} values were greater than the mean arterial P_{O_2} of 68 mmHg of birds at rest (Fig. 5) and, in other dives, equivalent to or even greater than start-of-dive P_{O_2} values (Ponganis et al., 2007). Similarly, final venous P_{O_2} values of some dives, especially short-duration dives, were not only greater than the mean venous value of penguins at rest but also greater than start-of-dive P_{O_2} (Ponganis et al., 2007).

Evidence for the maintenance of gas exchange during dives was also provided by the elevations of arterial/venous P_{O_2} and P_{N_2} recorded in the blood sampler data. In Table 1, arterial and venous P_{O_2} values at 1–3 min into dives were greater than or near the corresponding values of penguins at rest (Ponganis et al., 2007). In addition, during dives, mean arterial P_{N_2} rose to 2.1 ATA at depths of 25–32 m; this is again indicative of continued gas exchange during these shallow dives. During simulated dives, similar increases have been recorded in arterial P_{N_2} in Adelie penguins (*Pygoscelis adeliae*) and in venous P_{N_2} in king penguins (*Aptenodytes patagonicus*) (Kooyman et al., 1973; Ponganis et al., 1999).

The arterial P_{O_2} profiles and P_{N_2} values also provide insight into possible mechanisms affecting gas transfer in the lungs. Although

Table 1. Arterial and venous blood gas and O_2 content during dives of emperor penguins

Penguin	Site	P_{CO_2} pH	P_{O_2} (mmHg)	O_2 content (mmHg)	Time into dive (ml O_2 dl^{-1})	Sample depth (min)	Dive duration (m)	Maximum depth (min)	(m)
21	A	7.35	52	89	24.3	2.1	31-21	3.3	31
21	A	7.42	44	72	20.8	2.8	32-27	10.1	36
29	V	7.47	48	58	17.1	1.1	32	7.3	41
31	V	7.37	62	39	12.1	2.2	20-36	4.1	36
40	V	7.39	52	36	12.4	3.2	30	6.6	44

A, artery; V, vein. For comparison, mean arterial and venous pH, P_{CO_2}, P_{O_2}, and O_2 content of emperor penguins at rest (Ponganis et al., 2007) were 7.50 and 7.50, 42 and 49 mmHg, 68 and 41 mmHg, and 22.5 and 17.4 ml O_2 dl^{-1}, respectively. In birds 31 and 40, while they were at rest, venous P_{CO_2} values were 55 and 46 mmHg, and P_{O_2} values were 30 and 41 mmHg, respectively. All catheters were inserted in femoral vessels except for bird 31, in which the catheter was inserted into the axillary vein.

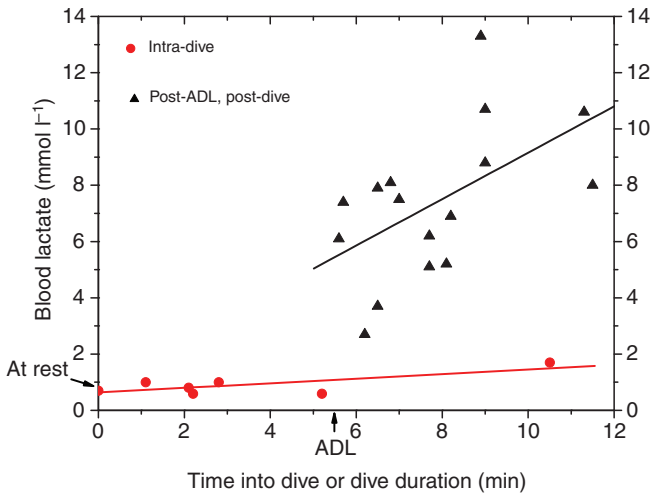

Fig. 10. Blood lactate concentrations as a function of time into dive or dive duration. Intradive blood lactate concentration was obtained using the blood sampler; post-dive lactate concentrations were measured previously (Ponganis et al., 1997).

the mean P_{N_2} of 2.1 ATA at 25–32 m depth is approximately twice the arterial value of penguins at rest, it is notable that the calculated air-sac P_{N_2} at these depths is 2.8–3.4 ATA, assuming an air-sac fraction of 0.8. The actual air-sac P_{N_2} is probably even higher since the O_2 fraction will decrease secondary to O_2 consumption (Kooyman et al., 1973). Thus, there appears to be an air sac-to-arterial difference not just for P_{O_2} (Fig. 4) but also for P_{N_2}. These differences may be due to ventilation–perfusion mismatch in the lung during the dive and/or due to the thickened parabronchial capillary blood-to-air barrier (Powell, 2000; Welsch and Aschauer, 1986). It is also notable that a large air sac-to-arterial difference in P_{O_2} has been observed in emperor penguins at rest (Ponganis et al., 2007). The typical increases in arterial P_{O_2} prior to the start of diving activity (see Fig. 3) suggest that the tachycardia and hyperventilation of the pre-dive period (Kooyman et al., 1971; Kooyman et al., 1992; Meir et al., 2008) improve ventilation–perfusion matching and account for these changes in P_{O_2} during the pre-dive period.

Despite air sac-to-arterial differences in P_{O_2} and P_{N_2}, the arterial P_{O_2} profiles and blood gas analyses obtained in this study all support the concept that pulmonary gas exchange is maintained during these shallow dives of emperor penguins with net transfer of O_2 from the respiratory O_2 store to the blood (Stockard et al., 2005).

Blood flow patterns

Implications for muscle blood flow during dives

Venous P_{O_2} profiles and intradive blood lactate concentration were first examined for evidence regarding muscle blood flow patterns during diving. We had hypothesized that a lack of muscle perfusion during dives would prevent muscle O_2 extraction from blood and also prevent the washout of lactate from muscle during dives longer than the ADL. In particular, despite the fact that buoyancy, stroke frequency and, therefore, muscle workload were highest during the initial descent period (Sato et al., 2002; van Dam et al., 2002), we expected the decline in venous P_{O_2} during the initial descent to be slow because of the hypothesized lack of muscle perfusion.

Although venous P_{O_2} profiles were variable in pattern and in overall rate of decline of P_{O_2}, 71% of dives actually had an increase in venous P_{O_2} during the initial descent period (Figs 6 and 7). In 15% of dives, venous P_{O_2} increased to values that were greater than the mean arterial P_{O_2} of birds at rest. The P_{O_2} of venous blood from which muscle has extracted O_2 should decline, not rise. Such increases in venous P_{O_2} during the initial descent period are thus not consistent with perfusion of active locomotory muscle. This hypothesis is further supported by dives in which final venous P_{O_2} values were greater than start-of-dive venous P_{O_2} values (see range of final P_{O_2} values for short-duration dives in Fig. 5) (see also Ponganis et al., 2007). In addition, blood lactate concentrations remained less than 2 mmol l^{-1} even as far as 10.5 min into a dive, well beyond the ADL (Fig. 10). Together, all these findings support a classical model of bradycardia and peripheral vasoconstriction in diving emperor penguins, in which muscle is isolated from the circulation during the dive, and lactate washout occurs during the eupneic period when muscle perfusion is re-established (Scholander, 1940; Scholander et al., 1942). This postulated lack of muscle perfusion during dives is also consistent with the lack of a relationship between stroke frequency and heart rate during dives of emperor penguins (Meir et al., 2008) and with increases in pectoral muscle temperature during dives of both emperor penguins and king penguins (Ponganis et al., 2003; Schmidt et al., 2007).

Implications for muscle blood flow before and after dives

Increased muscle perfusion and blood O_2 extraction during both the pre-dive period and the post-dive period were suggested by decreases in venous P_{O_2} during these times (see Fig. 6 as an example). Although venous P_{O_2} may decrease due to variety of factors, including O_2 consumption by tissues other than muscle, we suggest that these declines in venous P_{O_2} and previously documented pre-dive and post-dive tachycardias (Froget et al., 2004; Kooyman et al., 1992; Meir et al., 2008) support the concept of increased muscle perfusion and muscle O_2 extraction in both the pre-dive and post-dive period. Such pre-dive muscle hyperemia has also been postulated in king penguins because of decreases in muscle temperature prior to dives (Schmidt et al., 2006). If this hypothesis is correct, the declines in pre-dive venous P_{O_2} imply that muscle with high myoglobin content is not always fully saturated with O_2 during some rest periods and interdive intervals. This last suggestion is supported by the wide range of pre-submersion muscle O_2 contents in the early research of Scholander and colleagues (Scholander, 1940; Scholander et al., 1942). During the post-dive period, muscle reperfusion is, of course, expected. Venous P_{O_2} may not always decline during the post-dive period, however, because, even after muscle O_2 extraction from re-oxygenated arterial blood, the resulting venous P_{O_2} may still be greater than the end-of-dive venous P_{O_2}.

Implications for post-dive gas exchange

The return time of post-dive venous P_{O_2} (2.3 min) to the mean value of penguins at rest was slightly longer than the 1.9 min value for arterial P_{O_2}, but these times did not differ significantly. This was contrary to our hypothesis that O_2 uptake/consumption by muscle and other organs during the surface period would slow the replenishment of the venous blood O_2 store relative to the arterial rate. As expected, the return time (0.9 min) for air-sac P_{O_2} to reach the level at rest was significantly less than either of the blood values (ANOVA). Overall, the post-dive increases in air-sac, arterial and venous P_{O_2} values during the surface interval were rapid and consistent with prior reports of (1) a decrease in the post-dive tachycardia and resumption of a respiratory sinus arrhythmia within 2 min (Meir et al., 2008) and (2) a rapid decline in post-dive respiratory rate to levels of birds at rest in 3 min (Kooyman et al., 1971). These observations all suggest that the blood O_2 store is replenished and most CO_2 is exhaled within 3 min after a dive. The relationship of surface interval to the time for

post-dive venous P_{O_2} to reach 41 mmHg was not significant. A surface interval could be as much as 30 times longer than the time for venous P_{O_2} to return to a resting level. Although the time course of muscle re-oxygenation during the surface interval remains to be investigated, other factors such as sated appetites, food digestion, metabolic processing, social interactions and visits to the dive hole by Weddell seals probably also contribute to the duration of the surface interval of birds diving at the isolated dive hole.

Implications for arterio-venous shunts

The existence and function of arterio-venous shunts are often documented by elevations in venous P_{O_2} or O_2 content above control values or average values of animals at rest. Pre-dive venous P_{O_2} was greater than that of penguins at rest prior to 61% of dives. Arterio-venous anastamoses, which have been described anatomically in the extremities of birds (Arad et al., 1989; Thomas and Fordyce, 2007), may contribute to these elevations during surface periods. In addition, high blood flow through the vasculature of an extremity such as the wing may act as an effective shunt given the relatively low O_2 requirements of the bones, tendons and ligaments of the wing. Such flow through the wings and feet during the surface tachycardia is consistent with the re-warming previously recorded in the extremities during the surface period (Ponganis et al., 2003). In addition, this suggestion is supported by a brachial vein (in the wing) blood sample obtained during a surface interval. The P_{O_2} (81 mmHg) and P_{CO_2} (38 mmHg) not only were characteristic of arterial blood but, as would be expected during hyperventilation, also were above (P_{O_2}) and below (P_{CO_2}) the respective arterial values of penguins at rest. The similarity of the brachial vein P_{O_2} of 260 mmHg to that in the brachial artery during anesthesia on 100% O_2 was also consistent with arterio-venous shunting through the wing. Indeed, this mechanism probably contributes to the high venous P_{O_2} values reported during avian anesthesia (Jaensch et al., 2002). Therefore, we propose that arterio-venous shunting through the extremities during the post-dive period contributes to elevated pre-dive venous P_{O_2} values as well as to the rapid recovery of post-dive venous P_{O_2}.

Another site of arterio-venous shunting may be the brood patch vasculature. It has already been suggested that increased perfusion accounts for rewarming of the brood patch during surface periods of king penguins at sea (Schmidt et al., 2006). Since the brood patch contains arterio-venous anastamoses (Midtgard et al., 1985) and is not a site of high metabolic activity, increased flow in brood patch vessels could also contribute to elevations in venous P_{O_2}.

Although the relationship of dive duration to pre-dive or initial venous P_{O_2} was weak (R^2<0.1, Fig. 8), the potential significance of arterio-venous shunting during the surface period is exemplified by the pre-dive venous P_{O_2} of 63 mmHg prior to a 23.1 min dive, the longest dive ever reported for an emperor penguin. The Hb saturation, blood oxygen content and total blood O_2 store at that P_{O_2} should be considerably greater than the respective values at the mean venous P_{O_2} of 41 mmHg of birds at rest. Examination of the relationship of venous Hb saturation to dive duration awaits determination of the emperor penguin O_2–Hb dissociation curve.

The most remarkable and unexpected increases in venous P_{O_2} occurred in 78% of dives during early descent. In 15% of all dives, the peak venous P_{O_2} values were greater than the arterial value of birds at rest. Values could be as high as 90 mmHg (Fig. 6). Such high P_{O_2} values, which reflect arterial blood values and fully saturated Hb, suggest the existence of a significant arterio-venous shunt in addition to muscle ischemia during the dive. The elevated venous P_{N_2} values collected during the early parts of dives were also not significantly different from arterial values in the same depth range. This suggests minimal tissue uptake of N_2 and is again consistent with the existence of an arterio-venous shunt during the dive.

We propose that the blood flow through an arterio-venous shunt could be supported by the transient increase in heart rate that occurs during the early descent period of dives by penguins (Froget et al., 2004; Green et al., 2003; Meir et al., 2008). Therefore, as illustrated in Fig. 9, we hypothesize that a primary function of the transient increase in heart rate early in the dive is to enhance the transport of O_2 from the air sac and lungs into the arterial system and then, through a still-open arterio-venous shunt, into the venous system. This essentially represents a mechanism by which the size of the venous O_2 reservoir can be increased during the dive by transport of O_2 from the large respiratory O_2 store of the penguin into the blood. As such large increases in venous P_{O_2} were not always seen, the magnitude of such a shunt may vary in different dives. Indeed, the variability in venous P_{O_2} profile patterns during dives suggests that the vascular response may be quite plastic and adaptive to different conditions.

However, the anatomical basis of such an intradive arterio-venous shunt remains to be determined. Based on observations that bleeding from a nicked wing continued during the first 2.5 min of a dive of an emperor penguin (Kooyman et al., 1971), it is possible that blood flow through the wings contributes to these elevations in venous P_{O_2} during dives. If so, the countercurrent heat exchangers of the extremities (Arad et al., 1989; Thomas and Fordyce, 2007) are extremely efficient, given the preservation of core temperature and the significant cooling of both the wings and feet during dives (Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003). Conceivably, shunt flow may also occur in arterio-venous anastamoses described in the proximal regions of the extremities of birds (Arad et al., 1989). In addition, blood flow through the brood patch region could contribute to an arterio-venous shunt during a dive. It has already been suggested that such flow contributes to paradoxical increases in brood patch temperature during dives of king penguins (Schmidt et al., 2006). Another mechanism of arterio-venous shunting may involve the muscle vascular bed because avian muscle has been considered to have ‘luxuriant’, non-nutrient blood flow that contributes to higher than expected P_{O_2} in the venous effluent of muscle (Folkow et al., 1966; Grubb, 1981). In summary, there may be several anatomical sites for the hypothesized arterio-venous shunt during dives.

It should also be noted that it is unlikely that contraction of a large spleen and expulsion of arterialized blood into the venous system contribute to such elevations in venous P_{O_2} during dives of emperor penguins. First, we have never observed a large spleen in any of our many dissections of emperor penguin carcasses. Second, Hb concentration in three birds did not decrease during anesthesia or increase during diving in this study. Such changes would be expected if a large spleen dilated during anesthesia or contracted during diving activity as in seals (Hurford et al., 1996; Ponganis et al., 1993; Ponganis et al., 1992; Qvist et al., 1986). Third, blood introduced from the spleen should have a P_{N_2} near that at the surface and should delay the rise in venous P_{N_2} at depth, thus increasing, not decreasing, the arterial-to-venous difference in P_{N_2}.

CONCLUSIONS

Blood P_{O_2} profiles and analyses of blood samples during dives of emperor penguins are consistent with (a) maintenance of gas exchange between the air sacs, lungs and blood during dives, (b) muscle ischemia during dives, (c) muscle hyperemia both before and after dives, and (d) utilization of arterio-venous shunts during the surface period and during the dive. These physiological processes allow (1)

utilization of the large respiratory O_2 store of penguins, (2) regulated depletion of the blood O_2 store, (3) isolation of the respiratory and blood O_2 stores from working muscle, and (4) optimized loading of O_2 stores both before and after dives. The proposed muscle ischemia and the incomplete depletion of the respiratory and blood O_2 stores at the ADL suggest that muscle O_2 depletion and subsequent lactate accumulation represent the primary source of the post-dive increase in lactate at the ADL. The potential existence of an arterio-venous shunt during a dive was unexpected. Such increased flow through an arterio-venous shunt could be supported by the transient increases in heart rate previously reported during the early descent periods of dives.

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