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Returning on empty: extreme blood O_2 depletion underlies dive capacity of emperor penguins

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Summary

Blood gas analyses from emperor penguins (*Aptenodytes forsteri*) at rest, and intravascular P_{O_2} profiles from free-diving birds were obtained in order to examine hypoxemic tolerance and utilization of the blood O_2 store during dives. Analysis of blood samples from penguins at rest revealed arterial P_{O_2}s and O_2 contents of 68±7 mmHg (1 mmHg=133.3 Pa) and 22.5±1.3 ml O_2 dl^{-1} (N=3) and venous values of 41±10 mmHg and 17.4±2.9 ml O_2 dl^{-1} (N=9). Corresponding arterial and venous Hb saturations for a hemoglobin (Hb) concentration of 18 g dl^{-1} were >91% and 70%, respectively. Analysis of P_{O_2} profiles obtained from birds equipped with intravascular P_{O_2} electrodes and backpack recorders during dives revealed that (1) the decline of the final blood P_{O_2} of a dive in relation to dive duration was variable, (2) final venous P_{O_2} values spanned a 40-mmHg range at the previously measured aerobic dive limit (ADL; dive duration associated with onset of post-dive blood lactate accumulation), (3) final arterial, venous and previously measured air sac P_{O_2} values were indistinguishable in longer dives, and (4) final venous P_{O_2} values of longer dives were as low as 1–6 mmHg during dives. Although blood O_2 is not depleted at the ADL, nearly complete depletion of the blood O_2 store occurs in longer dives. This extreme hypoxemic tolerance, which would be catastrophic in many birds and mammals, necessitates biochemical and molecular adaptations, including a shift in the O_2–Hb dissociation curve of the emperor penguin in comparison to those of most birds. A relatively higher-affinity Hb is consistent with blood P_{O_2} values and O_2 contents of penguins at rest.

Key words: aerobic dive limit, blood gases, dive, emperor penguin, hypoxemia, P_{O_2}.

Introduction

Emperor penguins (*Aptenodytes forsteri*) are premier avian divers with routine dive durations of 5–12 min and a reported maximum dive duration of 22 min (Kooyman and Kooyman, 1995; Robertson, 1995). This breath-hold capacity is dependent, in part, on utilization and depletion of the blood O_2 store (Scholander, 1940). Since dissociation of all O_2 from hemoglobin (Hb) requires a low O_2 partial pressure (P_{O_2}), effective utilization of the blood O_2 store implies exceptional hypoxemic (low P_{O_2}) tolerance in these animals. Prior investigations of the diving physiology and behavior of emperor penguins at the corralled, isolated dive hole of our Penguin Ranch research camp on the sea ice of McMurdo Sound, Antarctica, have made such diving activity an ideal model in which to investigate blood O_2 depletion and hypoxemic tolerance.

While under the sea ice of McMurdo Sound, emperor penguins primarily feed on the sub-ice fish, *Pagothenia borchgrevinki* (Ponganis et al., 2000). Dive durations of 5–12 min are common, and dive depths are usually less than 100 m. During these dives, the birds undergo variable bradycardias (Kooyman et al., 1992), maintain aortic and vena caval temperatures near 37–39°C (Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003) and have an aerobic dive limit (ADL; dive duration associated with post-dive blood lactate accumulation) of 5.6 min (Ponganis et al., 1997b).

Particularly relevant to the current investigation was the recent successful application of an air sac P_{O_2} electrode and backpack recorder to diving birds at the Penguin Ranch (Stockard et al., 2005). P_{O_2} profiles obtained *via* the O_2 electrode revealed that 42% of these voluntary dives of emperor penguins had end-of-dive air sac P_{O_2} values less than 20 mmHg (1 mmHg=133.3 Pa). Such low P_{O_2} values are significant in comparison with other birds for several reasons. First, the lowest of these air sac values in emperor penguins is less than inspired air values (23 mmHg) of birds at altitudes as high as 11 580 m (Black and Tenney, 1980). Second, these values in free-diving emperor penguins are also lower than the air sac P_{O_2} values (~30 mmHg) of pekin ducks (*Anas platyrhynchos*) forcibly submerged to the point of 'imminent cardiovascular collapse' (Hudson and Jones, 1986). Third, because air sac P_{O_2} represents the maximum arterial P_{O_2} and, in fact, is usually greater than the simultaneous arterial value (Powell, 2000; Weinstein et al.,

1985), these low air sac values in emperor penguins imply that blood P_{O_2} values are commonly less than 20 mmHg. This is remarkable in that blood O_2 content at a P_{O_2} of 22 mmHg is very low (less than 5 ml O_2 dl^{-1} blood) in the high-altitude bird, the bar-headed goose (*Anser indicus*), and is even less in pekin ducks and pigeons (*Columbia livia*) (Black and Tenney, 1980; Hudson and Jones, 1986; Weinstein et al., 1985).

Therefore, in order to further investigate hypoxemic tolerance and blood O_2 depletion during dives, we equipped emperor penguins that were diving at the Penguin Ranch research camp with intravascular P_{O_2} electrodes and backpack recorders. In addition, we measured P_{O_2}, O_2 content, pH, P_{CO_2} and lactate concentrations in arterial and venous blood samples from birds at rest. Our goals were to determine (1) the baseline blood respiratory variables in birds at rest, (2) the relationship of the final blood P_{O_2} of a dive to dive duration, (3) the relationship of final blood P_{O_2} of a dive to the ADL, and (4) the hypoxemic tolerance of emperor penguins.

Materials and methods

General approach

In the austral springs of 2001 and 2003–2005, 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 six weeks, as in past studies (Kooyman et al., 1992; Ponganis et al., 1997b), and were then released at the McMurdo Sound ice edge. Blood-sampling catheters or P_{O_2} electrodes/thermistors were inserted percutaneously into the aorta or vena cava of emperor penguins under general isoflurane anesthesia as described previously (Ponganis et al., 1997b; Ponganis et al., 2001; Ponganis et al., 2004) and below. Catheterizations were confirmed as arterial or venous by observations of obvious pulsatile flows from arteries, by transduction of intravascular pressure and/or by P_{O_2} values of >250 mmHg in arteries and <100 mmHg in veins during anesthesia with 100% O_2 ventilation (Ponganis et al., 2004). After overnight recovery from anesthesia, birds were allowed to dive at the isolated dive hole. After 1–2 days of diving and data collection, probes and recorders were removed under general anesthesia. Data from the recorders were downloaded to a personal computer and analyzed with Microsoft Excel (OriginLab Corp., Northampton, MA, USA) and SPSS (SPSS, Inc., Chicago, IL, USA) software. Means are expressed ± standard deviation (s.d.). All procedures were approved under a UCSD Animal Subjects Committee protocol and US Antarctic Treaty Permit.

Catheterizations, blood samples at rest

For blood analyses in birds at rest, arterial samples were obtained *via* the femoral artery in one case and *via* the brachial artery in the wing in two cases. Venous samples were obtained from the femoral or axillary vein. The femoral artery was cannulated percutaneously with a 20 g epidural catheter (Perifix catheter; B. Braun Medical Inc., Bethlehem, PA, USA) *via* a 19 g thin-wall needle. Brachial arteries were catheterized percutaneously with 4.5 cm, 20 g catheters (RA-04020; Arrow International, Reading, PA, USA). Only short arterial catheters could be inserted in the wing secondary to the axillary arterial plexus in emperor penguins (Trawa, 1970). The femoral vein was catheterized percutaneously (Ponganis et al., 1999) with 5-Fr nylon catheters (Cook, Bloomington, IN, USA) or the 20 g epidural catheters *via* the same technique used for the femoral artery. In two cases, the axillary vein was catheterized with an epidural catheter *via* a 16 g catheter introducer (Becton Dickinson, Sandy, UT, USA) in the brachial vein of the wing. The femoral artery catheter and all venous catheters were inserted 15–30 cm into the aorta and vena cava, respectively. Catheters were flushed with heparinized (4 U heparin ml^{-1}) 0.9% saline. The portion of the catheter and stopcock external to the body was filled with a pre-measured volume of 40% ethanol/60% heparinized saline to prevent freezing of the external catheter solution (Ponganis et al., 1997b).

Blood samples from birds at rest were obtained between two and four hours after recovery from anesthesia. During this time, the birds were kept inside the penguin transport box (45×45×120 cm) near their corral at ambient Antarctic temperatures (–10 to –20°C). Distraction of the calm bird by one observer *via* the open lid of the box allowed the collection of the sample (after withdrawal of >3× the tubing deadspace) by a second researcher.

Blood gas (P_{O_2}, P_{CO_2} and pH) and lactate concentration analyses 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. O_2 content was determined with a Tucker chamber technique (Models SI 782 O_2 meter and 1302 O_2 electrode; Strathkelvin, Motherwell, Scotland, UK) (Tucker, 1967). Samples were analyzed within 10 min after collection. Blood gas, O_2 content and [lactate] were stable in the blood gas syringes (Model 4041, Sims Portex, Keene, NH, USA) for as long as 4 h at room temperature.

P_{O_2} electrode, thermistor, recorder

For P_{O_2} studies, a P_{O_2} electrode (Licox C1.1 Revoxode; Integra LifeSciences, Plainsboro, NJ, USA) (manufacturer's specifications: 90% response time <1 min, temperature correction factor <5% $°C^{-1}$, sensitivity error of <1%, and probe drift <2% day^{-1}) and thermistor (model 554; Yellow Springs Instruments, Yellow Springs, OH, USA) (60% response time 0.2 s, sensitivity 0.05°C), evaluated and calibrated as previously described (Ponganis et al., 2001; Ponganis et al., 2004; Stockard et al., 2005), were inserted 11–20 cm percutaneously into the vena cava or aorta *via* the femoral vein or artery with a peel-away introducer (PLIP 4.5 or 5.0-18-9-DENNY introducer; Cook) (Ponganis et al., 2001; Ponganis et al., 2004; Stockard et al., 2005). Prior to insertion, the P_{O_2} electrode was heparin-coated with an aseptic one-min immersion in 7% TDMAC heparin solution (Polysciences, Warrington, PA, USA).

In order to evaluate the effect of O_2 consumption by the electrode on O_2 depletion and a decline in P_{O_2} in stagnant blood, the output of two O_2 electrodes, each immersed in 2 ml of saline equilibrated with air at 38°C, was monitored when the equilibration air supply was shut off.

The custom P_{O_2}/temperature recorder (UFI, Morro Bay, CA, USA), protected in an underwater housing (250 g, 15×6×3.5 cm) and connected to the electrode and thermistor with waterproof cables (Sea Con, El Cajon, CA, USA; Impulse

Enterprise, San Diego, CA, USA), was mounted with a Velcro™ patch and cable ties as previously described (Stockard et al., 2005) while the bird was under anesthesia. It recorded both parameters at 15 s intervals. The bird was also equipped with a Mk9 time depth recorder (TDR, Wildlife Computers, Redmond, WA, USA) (sensitive to 0.5 m, 30 g, 6.5×1.7×1.7 cm; sample rate, 1 Hz) (Stockard et al., 2005).

Calibration and assay temperatures

In prior studies, mean aortic and vena caval temperatures during dives ranged from 38.3 to 39°C and from 37.2 to 38.3°C, respectively (Ponganis et al., 2004; Ponganis et al., 2003). Therefore, the output of the P_{O_2} electrode was temperature corrected to 38°C and all data in the P_{O_2} profiles are reported for a temperature of 38°C. For a P_{O_2} of 60 mmHg, a ±1°C temperature difference between an *in vivo* temperature and 38°C would result in a very minor ±4 mmHg difference between the *in vivo* P_{O_2} and the P_{O_2} profile reported at 38°C (Ashwood et al., 1983). For a P_{O_2} of 4 mmHg, the difference would be less than 0.3 mmHg.

Results of blood gas analyses are reported at 37°C because previously measured mean aortic and vena caval temperatures of emperor penguins at rest were 37.3–38.0°C and 36.3–38.7°C, respectively (Ponganis et al., 2004; Ponganis et al., 2003). For a P_{O_2} of 60 mmHg, P_{CO_2} of 40 mmHg and a pH of 7.40 reported at 37°C, an *in vivo* temperature of 38°C would again result in minimal changes in the *in vivo* values (Ashwood et al., 1983; Kiley et al., 1979), i.e. 64 mmHg, 42 mmHg and 7.39 pH units.

Results

Dive behavior

Histograms of dive duration and maximum depth in emperor penguins equipped with the intravascular P_{O_2} recorder are shown in Fig. 1. Arterial P_{O_2} profiles were obtained from 12 dives in three birds. Venous profiles were obtained from 130 dives in nine birds.

Blood analyses of emperor penguins at rest

Arterial P_{O_2}, O_2 content, pH, P_{CO_2} and [lactate] in three emperor penguins at rest were 68±7 mmHg, 22.5±1.3 ml O_2 dl^{-1}, 7.50±0.02 pH units, 42±6 mmHg and 1.0±0.2 mmol l^{-1}, respectively. Venous P_{O_2}, O_2 content, pH, P_{CO_2} and [lactate] in nine birds at rest were 41±10 mmHg, 17.4±2.9 ml O_2 dl^{-1}, 7.50±0.03 pH units, 49±4 mmHg and 0.7±0.3 mmol l^{-1}, respectively.

P_{O_2} profiles and final P_{O_2} values during dives

Arterial P_{O_2} profiles during dives were similar to those previously observed in the air sacs (Stockard et al., 2005). The rise and fall in air sac and arterial P_{O_2} profiles are illustrated in shallow dives of similar duration from two birds in Fig. 2. Final arterial P_{O_2} values, recorded during the last 15 s of dives as long as 6.8 min, ranged from 44 to 92 mmHg. The sample size of arterial P_{O_2} profiles (12 dives in three birds) was limited due to thrombus formation on the electrode, saltwater leaks into underwater connections and the technical difficulty of arterial catheterization.

Venous P_{O_2} profiles during a short dive and a long dive are illustrated in Figs 2 and 3. The final venous P_{O_2}, again recorded during the last 15 s of a dive, was obtained over a wide range of dive durations, including many dives beyond the ADL (Fig. 4A). Final venous P_{O_2} values reached values of 1–6 mmHg in some dives and were less than 20 mmHg in 29% of dives. An exponential regression was constructed from the highest final P_{O_2} during each 1-min interval of dive duration (Fig. 4A); it represents the minimum rate at which final venous P_{O_2} declined in relation to dive duration of emperor penguins at the isolated dive hole. The declines in final venous, arterial and air sac P_{O_2} (Stockard et al., 2005) in relation to dive duration are compared in Fig. 4B.

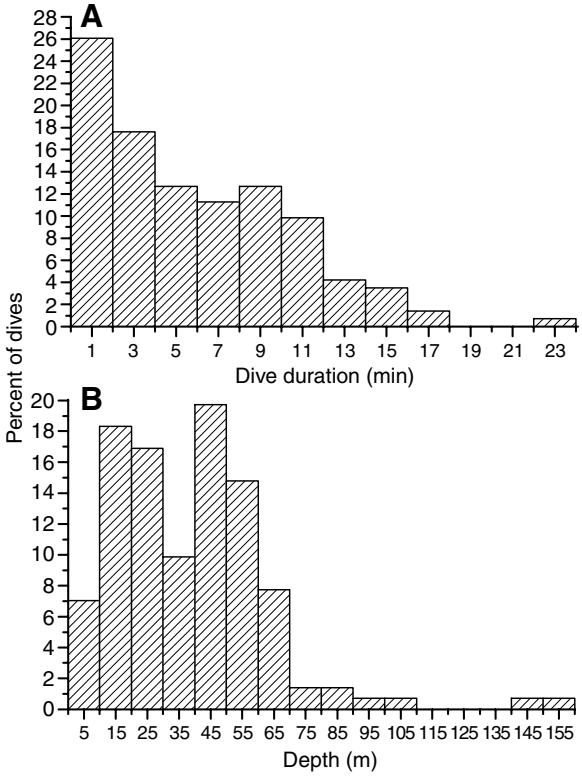

Fig. 1. Dive duration (A) and maximum depth (B) histograms of dives of emperor penguins equipped with backpack intravascular P_{O_2} recorders (*N*=142).

Blood temperatures during dives were similar to those recorded in previous studies (Ponganis et al., 2004; Ponganis et al., 2003). Final temperatures recorded during the last 15 s of dives ranged from 36.3 to 39.4°C.

In the test-tube evaluation of the depletion of O_2 in saline due to the O_2 consumption of the electrode, the P_{O_2} declined from 5 to 8 mmHg over the first 4 min after the equilibration air supply was shut off to the test tubes. It did not decline further over 20 min for either of the two P_{O_2} electrodes tested.

Discussion

Dive behavior

In emperor penguins equipped with the intravascular P_{O_2} recorder, dive durations and maximum depths were similar to those in past studies (Kooyman et al., 1992; Ponganis et al., 2001) at the isolated dive hole (Fig. 1). Dives of >100 m in depth were rare, and 47% of dives were >5.6 min, the

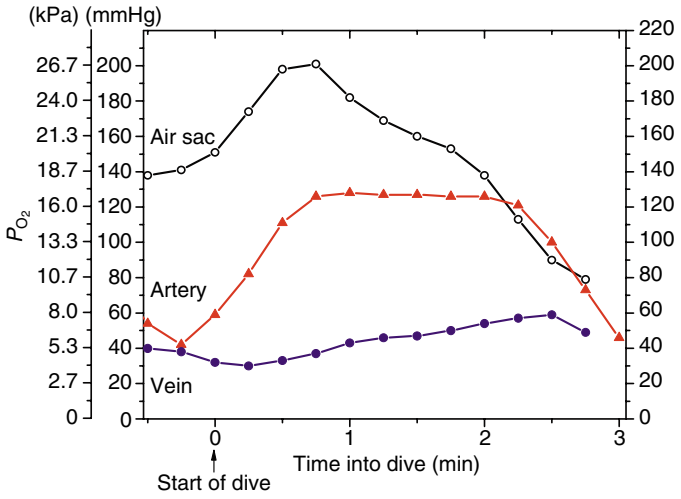

Fig. 2. Air sac, arterial and venous P_{O_2} profiles during shallow (less than 30 m), ~3-min dives in three different birds. P_{O_2} profiles from the air sac (EP 05) (Stockard et al., 2005) and aorta (EP 02) demonstrate initial compression hyperoxia and then a gradual decline in P_{O_2} secondary to air sac O_2 depletion and the decrease in ambient pressure during ascent. Vena caval P_{O_2} slowly increased during the dive of EP 20. The P_{O_2} data were recorded at 15 s intervals. Dives began at 0 time. Prior to the onset of the dive for the arterial profile, the bird had just surfaced for a breath after a prior dive.

previously measured ADL (Ponganis et al., 1997b). In 2004, birds often performed long-duration dives of >16 min; this included a 23.1 min dive (Fig. 3), which is now the longest reported dive in an emperor penguin. Dive profiles and lack of hunting ascents to the undersurface of the sea ice during these exceptional dives suggested that the birds were foraging at 50–80 m depth.

Blood analyses of emperor penguins at rest

In emperor penguins at rest, mean arterial P_{O_2} was 68±7 mmHg, less than two-thirds the mean value in the air sac of birds at rest (Stockard et al., 2005). Large air-sac-to-arterial differences in P_{O_2} in birds at rest are considered primarily due to ventilation–perfusion mismatch (Powell, 2000). It is unknown if this air-sac-to-arterial difference in P_{O_2} in emperor penguins at rest is also partially secondary to the thickened parabronchial capillary blood-to-air barrier that has been reported in these birds (Welsch and Aschauer, 1986).

Although these arterial P_{O_2} values in emperor penguins are in the lower range of values reported for birds at rest (Powell, 2000), the arterial O_2 content of 22.5±1.3 ml O_2 dl^{-1} still represented greater than 91% saturation of the previously measured average [Hb] of 18 g dl^{-1} (Kooyman and Ponganis, 1998). Arterial pH, P_{CO_2} and [lactate] in emperor penguins at rest were characteristic of other avian species (Powell, 2000) and are consistent with a lack of stress during the sampling procedure. Venous P_{O_2}, O_2 content, pH, P_{CO_2} and [lactate] were consistent with the observed arterial values and resulted in an estimated 70% venous Hb saturation.

P_{O_2} profiles and final P_{O_2} values during dives

Arterial P_{O_2} profiles reflected the effects of compression hyperoxia and O_2-store depletion previously documented in the air sacs during dives (Stockard et al., 2005). For example, during a 5.3-min, 60-m deep dive, arterial P_{O_2} increased from an initial value of 98 mmHg to a peak value of 257 mmHg and then gradually decreased to a final value of 76 mmHg near the end of the dive. Fig. 2 demonstrates the similarity of arterial and air sac P_{O_2} profiles in two different birds during shallow dives of short duration. Near the ends of some dives, the compression hyperoxia resulted in arterial P_{O_2} values that were greater than the mean value (68±7 mmHg) of birds at rest.

Venous P_{O_2} did not always simply decline during dives but sometimes increased (Fig. 2). This accounts for final dive values (Fig. 4A) that are greater than those of birds at rest. In addition, there was a wide range of pre-dive venous P_{O_2} levels (Figs 2 and 3). Prior to a 23.1 min dive (Fig. 3), the blood O_2 store was optimized with an elevated pre-dive venous P_{O_2} of 63 mmHg. This value was not only greater than that of birds at rest but was nearly equivalent to arterial values of birds at rest. These findings support the concept that the blood O_2 store of emperor penguins can be enhanced by ‘arterialization’ of venous blood.

Although final venous P_{O_2} declined in relation to dive duration, the relationship was variable; e.g. at a dive duration of 5.6 min (the ADL), final venous P_{O_2} values spanned a range of 40 mmHg. These final venous P_{O_2} data and the previously published final air sac P_{O_2} data provide evidence that the total body O_2 store is not depleted at the ADL. In fact, the body O_2 store is still not depleted even after many minutes

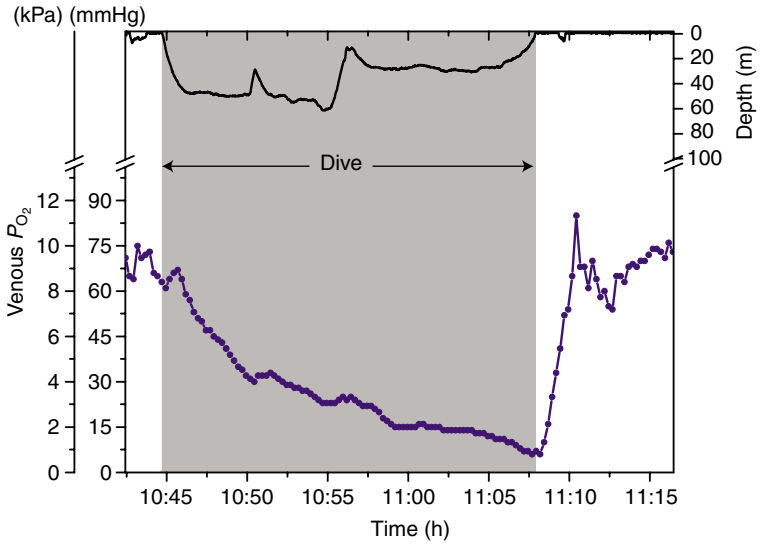

Fig. 3. Venous P_{O_2} and depth profiles from a 23.1 min dive. This shallow (<60 m maximum depth) dive is currently the longest reported dive of an emperor penguin. The blood O_2 store was optimized in this bird (EP 19) with a pre-dive venous P_{O_2} of 63 mmHg, which was equivalent to arterial values of birds at rest. P_{O_2} gradually declined throughout the dive to a final value of 6 mmHg and then returned to pre-dive levels within 3 min. Grey background indicates dive time.

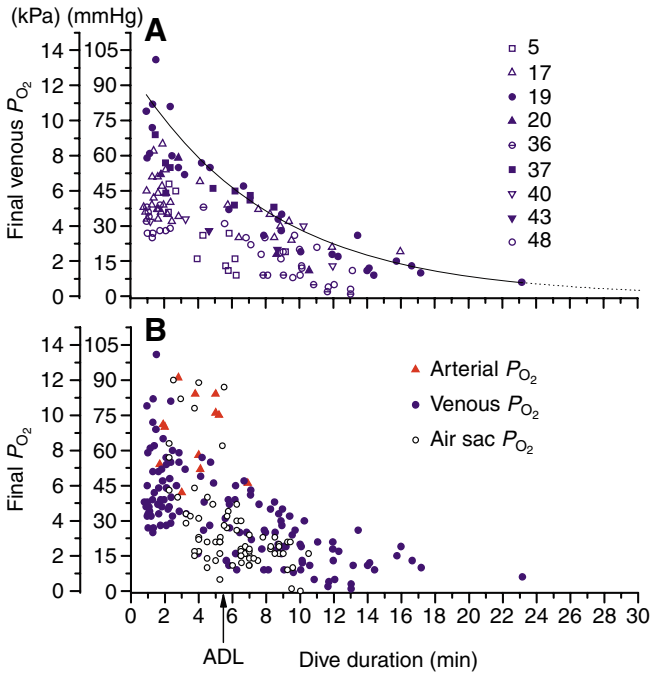

Fig. 4. Final P_{O_2} and dive duration. Final P_{O_2} values were recorded within the last 15 s of each dive. (A) Final venous P_{O_2} and dive duration from nine emperor penguins (key shows individual symbols for each penguin). The variability in final P_{O_2} values in relation to dive duration is presumably secondary to differences in metabolic rates during dives. In addition, the span of P_{O_2} values at the aerobic dive limit (ADL) clearly indicates that the blood O_2 store is not depleted at this limit, the dive duration associated with post-dive lactate accumulation. The exponential regression (y=96.416e$^{-0.1216x}$, r^2=0.94, P<0.001) was constructed from the highest final P_{O_2} during each 1-min interval of dive duration. This represents the minimum rate at which final P_{O_2} declines in relation to dive duration. (B) Comparison of final venous P_{O_2}, arterial P_{O_2} and air sac P_{O_2} (Stockard et al., 2005) demonstrates that air sac, arterial and venous P_{O_2} values become indistinguishable in longer dives.

beyond the ADL (Fig. 4). The wide range of final values for a given dive duration was consistent with variations in the rates of decline of P_{O_2} in the individual venous profiles and, presumably, was related to differences in rates of O_2 consumption during dives. The minimum rate at which final venous P_{O_2} declined in relation to dive duration of emperor penguins at the isolated dive hole is described by the exponential regression in Fig. 4A.

We also propose that venous P_{O_2} profiles and end-of-dive values, especially during the latter portions of long dives, approximate arterial values. Comparison of final air sac (Stockard et al., 2005) and venous P_{O_2} values from dives of emperor penguins reveals that final air sac values become indistinguishable from final venous values during longer dives (Fig. 4B). This is particularly apparent after dives beyond the ADL (Fig. 4B). Arterial P_{O_2} data, available over a limited range of relatively short dive durations, occupy the same range as air sac values and, in some cases, overlap venous values. Given the similar distributions of arterial, air sac and even some venous final P_{O_2} values for short dive durations, and the assumption that air sac P_{O_2} represents maximal arterial P_{O_2}, we think that venous final P_{O_2} values approximate arterial final P_{O_2} values for long dives. Similar equilibrations of venous and arterial P_{O_2} values have also been reported in seals (Elsner et al., 1964; Stockard et al., 2007). Thus, as indices of the entire blood O_2 store, the venous P_{O_2} profiles from the longer dives in this study demonstrate that emperor penguins clearly push the limits of hypoxemia and, indeed, are capable of 'returning on empty' to the dive hole. In 29% of dives, final venous P_{O_2} values were less than 20 mmHg; in some dives, P_{O_2} reached values as low as 1–6 mmHg. These final P_{O_2} values are well below the arterial and venous thresholds (20–25 mmHg) for cardiovascular collapse in pekin ducks (Hudson and Jones, 1986) and are also less than arterial P_{O_2} (22 mmHg) in bar-headed geese at 11 580 m altitude (Black and Tenney, 1980). In the 23 min dive of an emperor penguin, P_{O_2} was less than 20 mmHg for 8 min and eventually reached 6 mmHg (Fig. 3).

One might question whether the extremely low final venous P_{O_2} data could be secondary to O_2 consumption by the electrode in blood made stagnant by the bradycardia and low cardiac output of diving. We think this is unlikely for several reasons. First, low P_{O_2} values also occurred in the air sacs, which should not be affected by stagnant blood. Second, given the stroke volume and blood volume of emperor penguins (Kooyman et al., 1992; Ponganis et al., 1997a), even if heart rate were 5 beats min^{-1} during the last 10 min of the 23 min dive, the entire blood volume would circulate during that time period. Presumably, there would still be some flow past the electrode in that situation. Third, low final venous P_{O_2} values also occurred during short dives, which have higher heart rates (Kooyman et al., 1992) that should be associated with higher blood flows. Fourth, during the initial post-dive portion of the surface interval, venous P_{O_2} often stayed the same (Fig. 3) or even decreased further (P.J.P., unpublished data). This lack of an immediate increase in venous P_{O_2} during the tachycardia (Kooyman et al., 1992) and presumed high blood flows of the initial surface period again support our argument that the low values during dives are not secondary to the localized depletion of O_2 in a stagnant layer of blood around the electrode. Fifth, in the test-tube evaluation of the potential effect of the P_{O_2} electrode itself on O_2 depletion in saline, there was only a minimal initial decline in P_{O_2} over 4 min, and then no change thereafter. This change in P_{O_2} in saline should be the maximum potential effect of the electrode since localized O_2 depletion in blood would be buffered by release of O_2 from Hb. Therefore, we expect that the localized depletion of O_2 by the electrode in blood would be even less and that the low venous P_{O_2} values in this study are not secondary to O_2 consumption by the electrode.

These low P_{O_2} values in the blood and respiratory systems of diving emperor penguins are also remarkable in comparison to mammalian indices of hypoxemia, including (a) the typical arterial P_{O_2} criterion of 60 mmHg for treatment of human patients (Nunn, 1977), (b) end-tidal P_{O_2} values of 35 mmHg from climbers on ambient air at the top of Mount Everest (West et al., 1983), (c) human thresholds for shallow-water blackout near 25 mmHg (Ferrigno and Lundgren, 1999), (d) mixed venous P_{O_2} values of 27–34 mmHg in terrestrial mammals exercising at maximal O_2 consumption (Taylor et al., 1987), (e) femoral venous P_{O_2} values of 20 mmHg in humans exercising

at maximal O_2 consumption (Roca et al., 1992) and (f) arterial and end-tidal P_{O_2} values of 15–20 mmHg in free-diving Weddell seals (*Leptonychotes weddellii*) and bottlenose dolphins (*Tursiops truncatus*) (Ponganis et al., 1993; Qvist et al., 1986; Ridgway et al., 1969). The only P_{O_2} values equivalent to the extremes of hypoxemia found in these free-diving emperor penguins are the arterial and venous P_{O_2} levels (10 and 3 mmHg, respectively) found in harbor seals (*Phoca vitulina*) forcibly submerged to an electroencephalographic threshold for hypoxemic brain damage (Kerem and Elsner, 1973).

These findings of extreme hypoxemia in emperor penguins also suggest that, in contrast to the Hb of the pekin duck or pigeon (Hudson and Jones, 1986; Weinstein et al., 1985), the Hb of the emperor penguin is not stripped of all its O_2 at a P_{O_2} of 20 mmHg. In other words, the P_{50} (P_{O_2} at 50% O_2 saturation of Hb) of whole blood in emperor penguins is probably much lower than the P_{50} of pekin ducks (42–52 mmHg) (Black and Tenney, 1980; Lutz, 1980; Powell, 2000) and perhaps even lower than the P_{50} of isolated, reconstituted emperor penguin Hb (36 mmHg) (Tamburrini et al., 1994). Rather, it is probably closer to the lowest whole-blood P_{50} values (30–34 mmHg) found in Adelie, gentoo and chinstrap penguins (*Pygoscelis adeliae*, *P. papua*, *P. antarctica*) and in high-altitude-adapted birds such as the bar-headed goose (Black and Tenney, 1980; Milsom et al., 1973; Petschow et al., 1977). This suggestion of a relatively low P_{50} in the emperor penguin is supported by the blood gas and O_2 content analyses of these birds at rest. At a mean venous P_{O_2} of 41 mmHg, mean O_2 content was 17.4 ml O_2 dl^{-1}, which represents approximately 70% saturation of an average Hb concentration of 18 g dl^{-1} (Kooyman and Ponganis, 1998).

In comparison to the P_{50} of the pekin duck, a lower P_{50} in the emperor penguin would not only increase blood O_2 content during hypoxemia but it would also enhance dive capacity by allowing more complete depletion of the respiratory O_2 store. In the pekin duck forcibly submerged to the point of 'imminent cardiovascular collapse', 25% of the respiratory O_2 store was still unused because the blood contained almost no O_2 at an air sac P_{O_2} near 30 mmHg (Hudson and Jones, 1986).

In conclusion, intravascular/air sac P_{O_2} profiles in diving emperor penguins have revealed that their dive capacity is at least partially achieved through optimum management of the blood/respiratory O_2 stores and extreme hypoxemic tolerance. The blood P_{O_2} profiles provide insight into the nature and magnitude of physiological responses during the dive as well as into biochemical/molecular mechanisms underlying hypoxemic tolerance. In particular, a Hb with high O_2 affinity (low P_{50}) in penguins is essential not only to enhance blood O_2 content during hypoxemia but also to allow depletion of the respiratory O_2 store, which in emperor penguins constitutes 19% of the total body O_2 store (Kooyman and Ponganis, 1998). Other mechanisms of such extreme hypoxemic tolerance may include increased capillary densities, modifications in reactive O_2 species production and/or scavenging, and changes in the concentration and function of neuroglobin and cytoglobin. In addition to their relevance to the diving capacity and biology of emperor penguins, these potential cellular adaptations may also serve as models for improved understanding and treatment of human hypoxemic/ischemic pathologies.

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References

Ashwood, E. R., Kost, G. and Kenny, M. (1983). Temperature correction for blood gas and pH measurements. *Clin. Chem.* **11**, 1877-1885.

Black, C. P. and Tenney, S. M. (1980). Oxygen transport during progressive hypoxia in high-altitude and sea-level waterfowl. *Respir. Physiol.* **39**, 217-239.

Elsner, R. W., Scholander, P. F., Craig, A. B., Dimond, E. G., Irving, L., Pilson, M., Johansen, K. and Bradstreet, E. (1964). A venous blood oxygen reservoir in the diving elephant seal. *Physiologist* **7**, 124.

Ferrigno, M. and Lundgren, C. E. G. (1999). Human breath-hold diving. In *The Lung at Depth* (ed. C. E. G. Lundgren and J. N. Miller), pp. 529-586. New York: Marcel Dekker.

Hudson, D. M. and Jones, D. R. (1986). The influence of body mass on the endurance to restrained submersion in the Pekin duck. *J. Exp. Biol.* **120**, 351-367.

Kerem, D. and Elsner, R. W. (1973). Cerebral tolerance to asphyxial hypoxia in the harbor seal. *Respir. Physiol.* **19**, 188-200.

Kiley, J. P., Kuhlmann, W. D. and Fedde, M. R. (1979). Respiratory and cardiovascular responses to exercise in the duck. *J. Appl. Physiol.* **47**, 827-833.

Kooyman, G. L. and Kooyman, T. G. (1995). Diving behavior of emperor penguins nurturing chicks at Coulman Island, Antarctica. *Condor* **97**, 536-549.

Kooyman, G. L. and Ponganis, P. J. (1998). The physiological basis of diving to depth: birds and mammals. *Annu. Rev. Physiol.* **60**, 19-32.

Kooyman, G. L., Ponganis, P. J., Castellini, M. A., Ponganis, E. P., Ponganis, K. V., Thorson, P. H., Eckert, S. A. and LeMaho, Y. (1992). Heart rates and swim speeds of emperor penguins diving under sea ice. *J. Exp. Biol.* **165**, 161-180.

Lutz, P. L. (1980). On the oxygen affinity of bird blood. *Am. Zool.* **20**, 187-198.

Milsom, W. K., Johansen, K. and Millard, R. W. (1973). Blood respiratory properties in some Antarctic birds. *Condor* **75**, 472-474.

Nunn, J. F. (1977). *Applied Respiratory Physiology*. London: Butterworths.

Petschow, D., Wurdinger, I., Baumann, R., Duhm, J., Braunitzer, G. and Bauer, C. (1977). Causes of high blood O_2 affinity in animals living at high altitude. *J. Appl. Physiol.* **42**, 139-143.

Ponganis, P. J., Kooyman, G. L. and Castellini, M. A. (1993). Determinants of the aerobic dive limit of Weddell seals: analysis of diving metabolic rates, post-dive end tidal P_{O_2}'s, and blood and muscle oxygen stores. *Physiol. Zool.* **66**, 732-749.

Ponganis, P. J., Costello, M. L., Starke, L. N., Mathieu-Costello, O. and Kooyman, G. L. (1997a). Structural and biochemical characteristics of locomotory muscles of emperor penguins, *Aptenodytes forsteri*. *Respir. Physiol.* **109**, 73-80.

Ponganis, P. J., Kooyman, G. L., Starke, L. N., Kooyman, C. A. and Kooyman, T. G. (1997b). Post-dive blood lactate concentrations in emperor penguins, *Aptenodytes forsteri*. *J. Exp. Biol.* **200**, 1623-1626.

Ponganis, P. J., Kooyman, G. L., Van Dam, R. and Le Maho, Y. (1999). Physiological responses of king penguins during simulated diving to 136 m depth. *J. Exp. Biol.* **202**, 2819-2822.

Ponganis, P. J., Van Dam, R. P., Marshall, G., Knower, T. and Levenson, D. H. (2000). Sub-ice foraging behavior of emperor penguins. *J. Exp. Biol.* **203**, 3275-3278.

Ponganis, P. J., Van Dam, R. P., Knower, T. and Levenson, D. H. (2001). Temperature regulation in emperor penguins foraging under sea ice. *Comp. Biochem. Physiol.* **129A**, 811-820.

Ponganis, P. J., Van Dam, R. P., Levenson, D. H., Knower, T., Ponganis, K. V. and Marshall, G. (2003). Regional heterothermy and conservation of core temperature in emperor penguins diving under sea ice. *Comp. Biochem. Physiol.* **135A**, 477-487.

Ponganis, P. J., van Dam, R. P., Knower, T., Levenson, D. H. and Ponganis, K. V. (2004). Deep dives and aortic temperatures of emperor penguins: new directions for bio-logging at the isolated dive hole. *Mem. Natl. Inst. Polar Res. Special Issue* **58**, 155-161.

Powell, F. L. (2000). Respiration. In *Sturkie's Avian Physiology* (ed. G. C. Whittow), pp. 233-264. San Diego: Academic Press.

Qvist, J., Hill, R. D., Schneider, R. C., Falke, K. J., Liggins, G. C., Guppy, M., Elliott, R. L., Hochachka, P. W. and Zapol, W. M. (1986). Hemoglobin concentrations and blood gas tensions of free-diving Weddell seals. *J. Appl. Physiol.* **61**, 1560-1569.

Ridgway, S. H., Scronce, B. L. and Kanwisher, J. (1969). Respiration and deep diving in the bottlenose porpoise. *Science* **166**, 1651-1654.

Robertson, G. R. (1995). *The Foraging Ecology of Emperor Penguins (Aptenodytes forsteri) at two Mawson Coast Colonies, Antarctica*. Australia: Antarctic Division, Department of Environment, Sport, and Territories.

Roca, J., Agusti, A. G. N., Alonso, A., Poole, D. C., Viegas, C., Barbera, J. A., Rodriguez-Roisin, R., Ferrer, A. and Wagner, P. D. (1992). Effects of training on muscle O_2 transport at V_{O_2MAX}. *J. Appl. Physiol.* **73**, 1067-1076.

Scholander, P. F. (1940). Experimental investigations on the respiratory function in diving mammals and birds. *Hvalradets Skrifter* **22**, 1-131.

Stockard, T. K., Heil, J., Meir, J. U., Sato, K., Ponganis, K. V. and Ponganis, P. J. (2005). Air sac P_{O_2} and oxygen depletion during dives of emperor penguins. *J. Exp. Biol.* **208**, 2973-2980.

Stockard, T. K., Levenson, D. H., Berg, L., Fransioli, J. R., Baranov, E. A. and Ponganis, P. J. (2007). Blood oxygen depletion during rest-associated apneas of northern elephant seals (*Mirounga angustirostris*). *J. Exp. Biol.* **210**, 2607-2617.

Tamburrini, M., Condo, S. G., di Prisco, G. and Giardina, B. (1994). Adaptation to extreme environments: structure-function relationships in emperor penguin hemoglobin. *J. Mol. Biol.* **237**, 615-621.

Taylor, C. R., Karas, R. H., Weibel, E. R. and Hoppeler, H. (1987). Adaptive variation in the mammalian respiratory system in relation to energetic demand. II. Reaching the limits to oxygen flow. *Respir. Physiol.* **69**, 7-26.

Trawa, G. (1970). Note preliminaire sur la vascularisation des membres des spheniscides. *Oiseau Rev. Fr. Ornithol.* **40**, 142-156.

Tucker, V. A. (1967). Method of oxygen content and dissociation curves on microliter blood samples. *J. Appl. Physiol.* **23**, 410-414.

Weinstein, Y., Bernstein, M. H., Bickler, P. E., Gonzalez, D. V., Samaniego, F. C. and Escobedo, M. A. (1985). Blood respiratory properties in pigeons at high altitudes: effects of acclimation. *Am. J. Physiol.* **249**, R765-R775.

Welsch, U. and Aschauer, B. (1986). Ultrastructural observations on the lung of the emperor penguin (*Aptenodytes forsteri*). *Cell Tissue Res.* **2443**, 137-144.

West, J. B., Hackett, P. H., Maret, K. H., Milledge, J. S., Peters, R. M., Jr, Pizzo, C. J. and Winslow, R. M. (1983). Pulmonary gas exchange on the summit of Mt. Everest. *J. Appl. Physiol.* **55**, 678-687.