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PHYSIOLOGICAL RESPONSES OF KING PENGUINS DURING SIMULATED DIVING TO 136 m DEPTH

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Summary

To evaluate blood N_2 uptake and the role of the respiratory volume (air sacs/lungs) as a N_2 and O_2 reservoir in deep-diving penguins, diving respiratory volume (V_{DR}), heart rate (f_H), venous P_{N_2}, blood volume (V_b) and hemoglobin (Hb) concentration were measured in king penguins (*Aptenodytes patagonicus*) during forced submersions and compressions equivalent to depths up to 136 m. V_{DR} was 69±18 ml kg^{-1} (mean ± S.D.) in 62 submersions ranging from 4.4 atmospheres absolute (ATA; 1 ATA=101 kPa) (34 m) to 14.6 ATA (136 m). Submersion f_H averaged 30±7 beats min^{-1} (N=18), approximately 20 % of pre- and post-submersion values. Venous P_{N_2} values during and after submersions as deep as 11.2 ATA (102 m) were all less than 2.8 atmospheres N_2 (283 kPa) above ambient pressure, a previously measured threshold for symptomatic bubble formation. Mean V_b was 83±8 ml kg^{-1} (N=6); [Hb] was 17.6±0.7 g dl^{-1} (N=7). On a mass-specific basis, mean V_{DR}, and therefore total available N_2, is 41 % of that in shallow-diving penguin species. Total body O_2 stores, calculated from measured V_{DR}, V_b, [Hb], muscle mass and myoglobin concentration, are 45 ml kg^{-1}, with 23 % in the respiratory system. This small respiratory fraction in comparison with that in shallow-diving penguins suggests a lesser reliance on the respiratory oxygen store for extended breath-holding and also a reduced uptake of nitrogen at depth.

Key words: air sac, blood volume, dive, heart rate, haemoglobin, lung, N_2 stores, O_2 stores, pressure, king penguin, *Aptenodytes patagonicus.*

Introduction

Deep dives of any bird or mammal are associated with potential blood absorption of nitrogen from the respiratory system and the attendant risks of decompression sickness and N_2 narcosis (Kooyman and Ponganis, 1998). In pinnipeds, N_2 uptake is minimized during diving because of small diving lung volumes, lung compression and the forcing of lung air into cartilage-reinforced upper airways (Denison and Kooyman, 1973; Kooyman et al., 1970). In contrast, the volume of the air sacs and lungs (the respiratory volume) in diving penguins represents a potentially significant reservoir of N_2 and O_2 (Kooyman et al., 1973; Kooyman and Ponganis, 1990). Mean diving respiratory volume of Adelie (*Pygoscelis adeliae*) and gentoo (*P. papua*) penguins is 165 ml kg^{-1}, five times greater than that in pinnipeds (Kooyman et al., 1972, 1973). In addition, it has been stated that re-expansion of collapsed parabronchial air capillaries of birds would be highly unlikely if not impossible because of the sharp angles of curvature and high surface tension (Duncker, 1974). Therefore, lung compression does not appear to be a feasible mechanism for decreasing N_2 absorption in birds. Since arterial P_{N_2} was elevated in Adelie and gentoo penguins during simulated dives to depth, it was suggested that they avoid the risk of elevated P_{N_2} by making short-duration, shallow dives (Kooyman et al., 1973).

King (*Aptenodytes patagonicus*) and emperor (*A. forsteri*) penguins, however, frequently make dives to depths of 200 and 400 m, respectively (Kooyman et al., 1992a; Kooyman and Kooyman, 1995). How such deep-diving penguins avoid significant N_2 uptake is unclear, but several possibilites exist. Severe bradycardia and reduction in cardiac output could reduce the cumulative uptake of N_2 during diving. In addition, a pressure-induced restriction of gas exchange might also occur. Histological examinations of the lungs of emperor and Humboldt (*Spheniscus humboldti*) penguins have demonstrated thickened blood–air barriers and increased blood capillary volumes (Welsch and Aschauer, 1986; Maina and King, 1987). It has been postulated that, at depth, engorgement of the blood capillaries might fill the parabronchial air capillaries, thereby preventing or reducing gas exchange (Kooyman et al., 1999). However, both these hypotheses for decreased N_2 absorption would also result in decreased O_2 uptake from the respiratory system, which, in smaller penguins, contains 45 % of the total O_2 store (Kooyman and Ponganis, 1998). Thus, either mechanism would deprive the

penguin of access to a significant portion of the body O_2 store. To evaluate these hypotheses and to assess the magnitude of O_2 stores in deep-diving penguins, we measured diving respiratory volume (V_{DR}), heart rate (f_H), venous P_{N_2}, blood volume (V_b) and hemoglobin (Hb) concentration in king penguins during forced submersions to simulated depths of up to 136 m.

Materials and methods

Eight non-breeding adult king penguins (*Aptenodytes patagonicus* Gray), ranging in body mass from 9.5 to 13.1 kg, received brief training sessions (two sessions of five dives, of 1 to 4 min duration, over 2–3 h) in a pressure chamber at the biology laboratory on Ile de Possession, Crozet Archipelago, Antarctica. The submersion protocol and compression chamber were described by Kooyman et al. (1973). Compression and decompression rates were approximately equivalent to descent and ascent rates of $1\,m\,s^{-1}$. All procedures were approved by the UCSD Animal Subjects Committee and conducted under an Antarctic Conservation Act permit.

Diving respiratory volume was calculated on the basis of the volume of water required to compress the bird in the chamber from 1 atmosphere absolute (ATA; 1 ATA=101 kPa) to the maximum pressure of a given submersion (Kooyman et al., 1973). Correction for the compliance of the chamber with the restraint jacket and board inside was included in the calculation. No correction was made for air trapped in the feathers.

Heart rate was calculated from analysis of electrocardiograms (ECGs) recorded using Smart Heart software (Harley Street Software, Canada) on a notebook computer. Prior to experiments, subcutaneous ECG leads (Kooyman et al., 1992b) were placed under local anesthesia (2 % lidocaine) in free-standing, hooded birds. Pre-submersion, submersion and post-submersion f_H values were calculated by summing heart beats for 1–2 min pre- and post-submersion and dividing by the duration of the analyzed ECG segment.

Venous blood samples were obtained from three birds during chamber experiments. Percutaneous catheters (20 g, 1.1 mm outer diameter) were inserted into the wing veins of two birds and the femoral vein of one bird. The catheters extended 10–15 cm from the skin into the veins, and the catheter tips were probably near the proximal portion of the inferior vena cava. General anesthesia (isoflurane; Kooyman et al., 1992b) was administered for catheter placement; 4 h of recovery was allowed prior to experiments. Blood samples were analyzed for N_2 content using the Van Slyke technique for 1 ml samples of whole blood (Kooyman et al., 1973). As a control, N_2 contents of distilled water samples were within 3 % of published values (Altman and Dittmer, 1971). At the end of experiments, catheters and electrodes were removed under general or local anesthesia.

Plasma volume (V_p) was determined using the Evan's Blue dye technique (Castellini et al., 1987) in six birds, three from the chamber experiments and three additional birds. In the latter, catheterization was similar to that described above. Dye injection and sampling were conducted in four birds while they were awake and in two birds under general anesthesia. Hematocrit (Hct) was determined by microcentrifugation and [Hb] using a cyanmethemoglobin technique (Ames Mini-Pak Hb) in these six birds and one other bird. Blood volume was calculated as $V_p/(1–Hct)$.

Brain volume was estimated by measuring the cranial volume of six adult king penguin skulls found in the colony by filling the cranium with sand, weighing the sand, and dividing that value by the measured density of the sand.

Values are expressed as mean ± S.D. Analysis of variance and Tukey comparison of means were conducted using Statistica (Statsoft). Differences were assumed to be significant at $P<0.05$.

Results

Diving respiratory volume was determined for 62 submersions, ranging from 4.4 ATA (34 m) to 14.6 ATA (136 m). Mean V_{DR} was $69\pm18\,ml\,kg^{-1}$. The range of the mean V_{DR} values of the eight individual birds was $44–88\,ml\,kg^{-1}$.

Pre- and post-submersion f_H values for 18 submersions of six birds averaged 141±32 and 150±38 beats min^{-1}, respectively. Mean submersion f_H was 30±7 beats min^{-1}, significantly less than pre- and post-submersion f_H values. The durations of these submersions ranged from 2.5 to 5 min.

Mean venous N_2 content at surface ambient pressure, i.e. 1 ATA, was 1.12±0.08 vol % (N=9). As in prior studies (Kooyman et al., 1972, 1973), this N_2 content was assumed to be equivalent to a P_{N_2} of 0.79 atmospheres (1 atmosphere=101 kPa). Three venous blood samples obtained after less than 0.5 min at 4.4 ATA averaged 1.14±0.27 atmospheres N_2. The mean of four samples collected between 0.3 and 1.1 min at 7.8 ATA was 1.93±0.70 atmospheres N_2. Two samples obtained after 0.3 and 1.1 min at 11.2 ATA were 1.50 and 2.21 atmospheres N_2, respectively. Two post-submersion samples (2.63 and 1.44 atmospheres N_2) were collected 10 and 40 s after submersions to 7.8 ATA.

Mean V_p and V_b of six birds were $46\pm4\,ml\,kg^{-1}$ and $83\pm8\,ml\,kg^{-1}$, respectively. The body mass of these birds was 11.7±1.2 kg. Mean [Hb] and Hct in seven penguins were $17.6\pm0.7\,g\,dl^{-1}$ and 45±2 % respectively. There were no significant differences in V_p or Hct between anesthetized and awake birds.

The cranial volume of six skulls was 28±2 ml. This is considered to be a maximum estimate of brain volume.

Discussion

The air volumes measured in these experiments include any plumage air in addition to the V_{DR}. Plumage volumes under these conditions are probably less than in the unrestrained situation because restrained birds are unable to preen. Past

experiments (Kooyman et al., 1973) demonstrated that plumage air of Adelie penguins under these conditions was not more than 10 % of the total air volume measured (or approximately 15 ml kg^{-1}). Thus, the actual V_{DR} of these king penguins during forced submersions is probably less than 69 ml kg^{-1}. Offsetting this lower value is the possibility that the V_{DR} would be greater during unrestrained conditions, as it is in the lesser scaup *Aythya affinis* (Stephenson, 1995). Nonetheless, it is notable that, under similar experimental protocols, the mass-specific V_{DR} in king penguins is 41 % of that of gentoo and Adelie penguins. This represents a significantly smaller potential N_2 and O_2 reservoir in king penguins.

It is not known whether pulmonary gas exchange continues in penguins regardless of depth during dives. However, if gas exchange is not restricted, the maximum available respiratory N_2 in a typical 12 kg king penguin is approximately 700 ml, assuming a V_{DR} of 69 ml kg^{-1} and a respiratory N_2 fraction of 0.9 (Kooyman et al., 1973). If dives were deep enough and long enough, and if all this N_2 were absorbed and distributed in the total body water of a 12 kg king penguin, the blood P_{N_2} could theoretically reach approximately 290 kPa or 2.9 atmospheres (for assumptions and calculations, see Kooyman and Ponganis, 1990). This P_{N_2} is 38 % of the value that would result if the mass-specific V_{DR} of Adelie and gentoo penguins were used in the calculations. It is also below the threshold for symptomatic bubble formation in cats (Harvey et al., 1944). At the opposite theoretical extreme, blood P_{N_2} could be greater than 4700 kPa (47 atmospheres) in a king penguin if this maximum N_2 gas volume were completely absorbed but limited by restricted peripheral blood flow to distribution to the blood volume (1 l), heart tissue (84 ml; Drabek, 1988) and brain (28 ml).

Although maximum arterial P_{N_2} during diving potentially equals that in the parabronchial airways (in the absence of a shunt or restricted gas exchange), the total volume of N_2 absorbed will also be a function of the magnitude and distribution of cardiac output during a submersion. In these experiments, f_H profiles were characteristic of forced submersion responses (Scholander, 1940). Mean f_H during the entire submersion period was 30 beats min^{-1}, approximately 50 % of that recorded in free-diving emperor penguins (Kooyman et al., 1992b). Presumably, cardiac output was correspondingly reduced in the king penguins, and blood flow was predominantly restricted to the brain, heart and lungs. In this situation, although the arterial P_{N_2} would be maximized, the total amount of N_2 absorbed would be limited by the low pulmonary flow and the small volume of distribution. During periods of decreasing pressure (i.e. during ascent and surface tachycardias), increased flow, increased volume of distribution (i.e. splanchnic organs and muscle not saturated with N_2 as a result of prior vasoconstriction) and N_2 exchange into the respiratory system should decrease blood P_{N_2}. The low venous P_{N_2} values during and after submersions are consistent with this model of low N_2 uptake and subsequent redistribution of N_2. No blood P_{N_2} values were greater than 2.8 atmospheres above ambient pressure, the threshold for symptomatic bubble formation in cats (Harvey et al., 1944). This model is also supported by the prior data of Kooyman et al. (1973), in which arterial P_{N_2} decreased significantly in blood samples obtained after decompression but prior to the first breath after simulated dives of Adelie penguins. Thus, in the absence of restricted pulmonary gas exchange at depth, possible roles of a bradycardia during diving and a tachycardia during ascent may be (1) to limit cumulative uptake of N_2 and (2) later to maximize its distribution during periods of decreasing pressure.

Our venous P_{N_2} data do not allow complete evaluation of the possible restriction of gas exchange in king penguins at depth by the hypothesized engorgement of parabronchhial blood capillaries. A similar mechanism of blood capillary volume expansion presumably prevents 'lung squeeze' in humans diving to depths at which lung volume would be compressed below residual volume (Craig, 1968). Future studies of peak arterial P_{N_2} are still required to evaluate this possibility in penguins. Our venous P_{N_2} data are consistent with this model, but may not reflect peak arterial values during submersion because of venous admixture and tissue N_2 uptake.

The relatively small V_{DR} of king penguins also affects the distribution of O_2 stores. The V_{DR}, V_b and [Hb] data from this study, previous muscle mass and myoglobin determinations (Cherel et al., 1993; Baldwin et al., 1984) and the O_2 saturation and extraction assumptions used in past calculations (Kooyman and Ponganis, 1990) allow the most accurate available estimation of O_2 stores for any penguin. These data yield a total body O_2 store of 45 ml kg^{-1}, with 23 % in the respiratory system, 30 % in the blood and 47 % in muscle. This respiratory O_2 store is smaller than prior estimations for king penguins and is 50 % less than the value in smaller penguins (Kooyman and Ponganis, 1998). It is similar to that in otariid pinnipeds (Kooyman, 1989). The resulting greater O_2 distribution in blood and muscle of king penguins makes reliance on respiratory O_2 stores less critical during diving. This is advantageous if blood N_2 uptake and also O_2 uptake are limited during deep dives. It is also notable that the Hb concentrations and mass-specific blood volumes of king penguins and emperor penguins (Ponganis et al., 1997) are in the same range as those of Adelie penguins (Lenfant et al., 1969). Therefore, the results of the present study indicate that king penguins have adapted to deep diving by (1) a reduction in respiratory O_2 stores, (2) a relative increase in muscle O_2 stores, and (3) a reduction in respiratory N_2 uptake, possibly secondary to either reduced cardiac output or a pressure-induced restriction of pulmonary gas exchange. Similar adaptations also probably function in emperor penguins, which display diving patterns similar to those of king penguins, but of nearly twice the depth and duration.

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